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Health Assessment Document for Epichlorohydrin

Final Report

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16. ABSTRACT

This document summarizes current scientific information regarding the health and environmental effects of epichlorohydrin. Specifically, this document discusses the following topics: physical and chemical properties of epichlorohydrin; environmental occurrence; metabolism and toxicology; and factors most relevant to assessing quantitative health risks, particularly risk of cancer. Epichlorohydrin is moderately toxic by oral, dermal, and inhalation routes of exposure. Long-term animal studies provide evidence that epichlorohydrin is a weak contact carcinogen which appears to produce no metastases. Epichlorohydrin should be considered a potential human carcinogen (IARC category 2B). Epichlorohydrin causes gene and chromosomal mutations *in vitro* and *in vivo*, and has been shown to be clastogenic in human lymphocytes. Epichlorohydrin was not teratogenic in mice, rats, or rabbits, but was embryotoxic at doses which were toxic to the pregnant mouse. Transient infertility was observed in male rats exposed to epichlorohydrin. Epichlorohydrin is not expected to persist in air, water, or soil because of hydrolysis. The atmospheric residence time was calculated to be 5.8 days.

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a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

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PREFACE

The Office of Health and Environmental Assessment has prepared this health assessment to serve as a "source document" for Agency-wide use. The health assessment document was originally developed at the request of the Office of Air Quality Planning and Standards; however, the scope of the assessment has since been expanded to address multimedia aspects. This assessment will help ensure consistency in the Agency's consideration of the relevant scientific health data associated with epichlorohydrin.

In the development of the assessment document, the scientific literature has been inventoried, key studies have been evaluated and summary/conclusions have been prepared so that the chemical's toxicity and related characteristics are qualitatively identified. Observed effect levels and other measures of dose-response relationships are discussed, where appropriate, so that the nature of the adverse health responses are placed in perspective with observed environmental levels.

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The EPA Office of Health and Environmental Assessment (OHEA) is responsible for the preparation of this health assessment document. The OHEA Environmental Criteria and Assessment Office (ECAO/RTP) had overall responsibility for coordination and direction of the document preparation and production effort. The chapters addressing physical and chemical properties, sampling and analysis, and toxicity data were written by Theodore Keneklis, Ph.D., Lawrence Kaufman, Ph.D., William McLellan, Ph.D., Nicholas Mujjar, Ph.D., Cipriano Cueto, Ph.D., and John Strange, Ph.D., all of Dynamac Corporation.

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1. EXECUTIVE SUMMARY

1.1 BACKGROUND INFORMATION

1.1.1 Properties

Epichlorohydrin (1-chloro-2,3-epoxypropane) is a colorless liquid with a characteristic chloroform-like, irritating odor. It is partially miscible with water and soluble in benzene, alcohol, and ether. It is a bifunctional alkylating agent that can chemically bind with many cell constituents. The epoxy group of epichlorohydrin is highly reactive. In most reactions, the compound behaves primarily as an epoxide, initially combining through the epoxy group to form 3-chloro-2-hydroxypropyl derivatives. Epichlorohydrin undergoes a variety of chemical reactions with many compounds, and thus is widely used as a chemical intermediate.

1.1.2 Production

Epichlorohydrin is produced commercially by high temperature chlorination of propylene to allyl chloride, followed by chlorohydration with hypochlorous acid to form a mixture of isomeric glycerol dichlorohydrins. The mixture is subsequently dehydrochlorinated with alkali to yield epichlorohydrin. Epichlorohydrin is produced in the United States by the Dow Chemical Company and Shell Oil Company. U.S. production in 1977 was 276 million pounds (134 million kilograms). In 1982, 330 million pounds were produced.

1.1.3 Use

Epichlorohydrin's major use is as a constituent of epoxy resins and glycerol. Epichlorohydrin is also used as a raw material for the manufacture of glycerol and glycidol derivatives used as plasticizers, stabilizers, solvents, dyestuff intermediates, surface active agents, and pharmaceuticals. It is also used in such products as paints, varnishes, and shellacs. In addition, it is used directly as a stabilizer in chlorine-containing materials such as synthetic rubber and certain insecticides.

1.1.4 Environmental Release, Transport, and Fate

The largest sources of emission of epichlorohydrin to the environment are from its manufacture, use as an intermediate, or from accidental spills. The ultimate environmental fate of epichlorohydrin depends on its release, transport, and persistence characteristics. Epichlorohydrin is known to be released into (1) the atmosphere from manufacture and use, (2) water from industrial effluents, and (3) the terrestrial compartment from spills and dumping. Epichlorohydrin is not expected to persist in air, water, or soil because of its tendency to hydrolyze and otherwise degrade. If released at the water/soil interface, epichlorohydrin's water solubility, estimated soil adsorption coefficient, and theoretical behavior in a landfill indicate the compound will enter the water. Epichlorohydrin released at the air/soil interface will enter the air because of the compound's high volatility and soil mobility. At the air/water interface, epichlorohydrin will partition into both media.

1.1.5 Environmental Transformation

In the environment, the major chemical transformation of epichlorohydrin is through hydrolysis; the half-life in distilled water at 20^{0} C is 8 days. Hydrolysis is expected to be faster if chloride or carbonate-bicarbonate ions are present. The major hydrolysis product of epichlorohydrin is 3-chloro-1,2-propanediol. Other possible transformation processes in the environment are photolysis and oxidation, but these would be minor compared to hydrolysis.

1.2 UPTAKE, METABOLISM, AND EXCRETION

Epichlorohydrin is readily absorbed and rapidly distributed to various tissues and organs. In laboratory mammals, the highest concentrations after exposure were found in the kidney, liver, pancreas, adrenals, and spleen. Following an oral dose of ^{14}C-epichlorohydrin to rats, the compound was rapidly absorbed from the gastrointestinal tract. The major routes of elimination in rodents were via the kidneys and lungs. Approximately 40 percent of the radioactivity, regardless of the route of administration, was excreted in the urine within 72 hours, and about 20 percent was exhaled as ^{14}C- carbon dioxide. Fecal excretion amounted to about 4 percent of the dose. Epichlorohydrin is metabolized first by hydrolysis, then by oxidation to oxalic acid or by conjugation with glutathione to form mercapturic acid derivatives.

1.3 EFFECTS ON HUMANS

Epichlorohydrin as a liquid or vapor can cause respiratory, skin, and eye irritation in humans. Pulmonary and liver changes were detected following exposure in one case study. Headache, nausea, and head and chest congestion were reported following the worker's exposure to epichlorohydrin. Local skin contact with epichlorohydrin is reported to cause severe skin irritation. Severe skin burns as well as burning of the eyes have occurred following accidental exposures. Allergic reactions have also been reported in workers occupationally exposed to epichlorohydrin. In one case of a severe epichlorohydrin inhalation exposure, initial irritation of the eyes and throat was followed by chronic asthmatic bronchitis. In this poisoning case, liver biopsies showed extensive fatty infiltration and degenerative changes. 1.4

1.4 ANIMAL TOXICITY

Epichlorohydrin is well absorbed and moderately toxic by oral, dermal, and inhalation routes. The acute oral dose lethal to 50 percent of rats exposed (LD_{50}) to epichlorohydrin was approximately 250 mg/kg body weight. The inhalation 6-hours LC_{50} in rats was 360 ppm, and the no-observed effect level (NOEL) was 283 ppm for 6 hours. Acute exposure caused central nervous system depression and death resulting from respiratory paralysis. A single nonlethal dose can cause kidney and lung damage in rats. Subchronic exposure by inhalation, oral, and intraperitoneal injection routes studies caused severe renal toxicity. Epichlorohydrin was intensely irritating to skin, nasal mucosa, and eyes; in addition, it can cause skin sensitization in laboratory animals. The target organs or tissues, listed in descending order of sensitivity to epichlorohydrin, are the nasal mucosa (when inhaled), kidneys, liver, and cardiovascular system. There was no unique strain or species sensitivity indicated.

1.5 CARCINOGENICITY, MUTAGENICITY, AND REPRODUCTIVE AND TERATOGENIC EFFECTS

1.5.1 Carcinogenicity

Results of long-term animal studies provide sufficient evidence of the carcinogenic potential of epichlorohydrin as a weak contact carcinogen which appears to produce no metastases. It is both route-dependent and site-specific in that tumors appear only at the site of first contact. In view of increases in nasal carcinomas seen in rat inhalation studies, the increased forestomach tumors in rat drinking water and gavage studies, the increased local sarcomas produced in mice after subcutaneous injection of epichlorohydrin, and the chromosomal aberrations found in the peripheral lymphocytes of exposed workers, epichlorohydrin should be considered a potential human carcinogen. Although three epidemiologic studies have not demonstrated epichlorohydrin to be carcinogenic to humans, they cannot be regarded as indications that epichlorohydrin is safe because the studies had some inherent weaknesses. Considering the above evidence and applying the International Agency for Research on Cancer (IARC) approach (Appendix F) for classifying the weight of evidence for carcinogenicity in experimental animals, epichlorohydrin would be placed in the 2B category, meaning that it is probably carcinogenic to humans. The 95 percent upper-limit incremental unit risk estimate for continuous inhalation exposure to 1 μg epichlorohydrin/m^3 of air is 1.2×10^{-6} based on extrapolation from the rat inhalation study. In terms of continuous exposure to 1 ppm, the upper-limit unit risk is 4.8×10^{-3}. For exposure via drinking water, the 95 percent upper-limit incremental unit risk estimate from water containing 1 µg epichlorohydrin per liter is 2.8×10^{-7}, based on an animal drinking water study. In terms of relative carcinogenic potency, epichlorohydrin exposure by either route is among the weakest of the chemicals that the EPA has evaluated as suspect carcinogens.

1.5.2 Mutagenicity

Substantial evidence is available demonstrating that epichlorohydrin causes gene and chromosomal mutations in several experimental systems both *in vitro* and in animals. Cytogenetic studies of workers exposed to epichlorohydrin have yielded evidence for a clastogenic effect on lymphocytes. The compound has been shown to be an active inducer of gene mutations in bacteria, yeast, *Drosophila*, and cultured mammalian cells. Epichlorohydrin is also effective in causing sister chromatid exchanges in human cells *in vitro* and preferential cell killing of repair-deficient bacteria. Chromosomal effects induced by epichlorohydrin were detected in both *in vivo* and *in vitro* mammalian cell assays.

It may be hypothesized that epichlorohydrin mutagenic action results from its alkylating reactivity. Epichlorohydrin should be considered as potentially hazardous to humans because of its clastogenic action in experimental systems.

1.5.3 Reproductive and Teratogenic Effects

Results from published studies indicate that epichlorohydrin (under the conditions of the studies) was not teratogenic in mice, rats, or rabbits. Signs of embryotoxicity were observed at doses that were toxic to the pregnant mouse. Transient infertility was observed in male rats exposed to epichlorohydrin, and recovery followed termination of exposure. No detrimental effects were observed on the fertility of male workers exposed to epichlorohydrin; however, weaknesses in study design prevent conclusions concerning the potential for causing infertility.

1.6 SYNERGISM AND ANTAGONISM

Synergistic and antagonistic relationships at the physiological level between epichlorohydrin exposure and other variables (cholesterol ingestion, cold stress, and heat stress) were limited to a few fragmentary studies. Rabbits ingesting cholesterol and epichlorohydrin (but not those consuming epichlorodydrin alone) showed impaired heart function and increased blood lipid levels. Rats inhaling a single 4-hour dose of epichlorohydrin followed by a cold stress (5^0 C for 2 hours) showed very few physiological differences from rats that were not cold-stressed. On the other hand, rats subjected to heat stress (35^0 C for 2 hours/day for 4 weeks) and epichlorohydrin inhalation (4 hours/day for 4 weeks) showed enhanced toxicity.

1.7 ECOSYSTEMS AND AQUATIC BIOTA

No studies were found that discussed the effects of epichlorohydrin on ecosystems. Toxicity data available for bacteria, algae, protozoa, aquatic invertebrates, and fish indicate that neither growth inhibition nor mortality in aquatic biota would occur at aqueous environmental concentrations of epichlorohydrin below 5 mg/l (5 ppm). Environmental levels as high as 5 ppm have not been shown to occur in the natural environment. Calculated estimates indicate that low levels of epichlorohydrin, which may potentially occur in the environment, would not pose significant bioconcentration or bioaccumulation hazards in the food chain.

1.8 REGULATIONS AND STANDARDS

Epichlorohydrin is currently controlled by U.S. and foreign regulations. U.S. regulations provide exposure limits in the workplace, restrictions on use in food and related industries, discharge limits into navigable waters, transportation procedures, and maximum disposal limits requiring special landfills. Epichlorohydrin is not currently regulated under the Safe Drinking Water Act or the Clean Air Act.

1.9 CONCLUSIONS

Epichlorohydrin is a potential hazardous compound because of its alkylating properties, its mutagenicity in a variety of systems, and its probable carcinogenic potential for humans as determined by animal bioassay data. Moreover, increased chromosomal aberrations have been reported in peripheral lymphocytes of workers exposed to epichlorohydrin. Further studies are needed for a more definitive conclusion on the possible effects of epichlorohydrin on humans.

1.10 RESEARCH NEEDS

Research needed to support or strengthen the existing data base on epichlorohydrin are indicated in the following section. Particular emphasis is placed on areas of studies needed to assess more fully the health hazards of human exposure to epichlorohydrin.

Epidemiology:

- Prospective and retrospective cohort studies of exposed workers should be pursued with special attention given to quantification of individual exposure levels versus health effects.

- Monitoring the lymphocytes of workers exposed to epichlorohydrin for cytogenetic damage should be continued. These studies should include sister-chromatid exchange analysis and a suitable assay for mutations in somatic cells. Health monitoring should include analysis of changes in blood count and should take into account smoking and drinking habits. The occurrence and frequency of sperm-morphology changes among exposed workers should be studied.

- Kidney function monitoring (e.g., BUN, creatinine, protein) should be done periodically for exposed epichlorohydrin workers to determine whether any changes in kidney function are occurring as a result of occupational exposure to the chemical.

- The hemoglobin alkylation technique should be applied to workers currently exposed to measured levels of epichlorohydrin to determine if the number of immature erythrocytes present in the peripheral blood increase with increased exposure.

Subchronic and Chronic Toxicity:

- Chronic oral and inhalation exposure tests on mammals at several concentrations of epichlorohydrin up to the maximum tolerated concentration (6 hours/day, 5 days/week for 2 years) are needed.

- Since the nasal mucosa and kidneys appear to be two of the most sensitive tissues to epichlorohydrin, mechanisms leading to mucosal and renal lesions should be explored.

- Since epichlorohydrin induces kidney and liver damage in mammals, studies should be conducted to determine if hypertension is also induced or aggravated by epichlorohydrin. Accordingly, the effects of epichlorohydrin on the heart should be more thoroughly investigated.

Genetic Toxicity:

- Mammalian studies using cytogenetic analysis of mouse bone marrow should be carried out following epichlorohydrin exposure by the inhalation route.

- A carcinogenic bioassay of epichlorohydrin in mammals exposed by the oral and respiratory routes at several dose levels up to the maximum tolerated dose should be conducted. The study should be designed to serve as a model for assessment of carcinogenic risk to humans.

Reproductive and Teratogenic Research:

- Since the acidity of the stomach may lead to hydrolysis of epichlorohydrin, reproductive and teratogenic effects might be observed if a mode or route of dosing other than gastric intubation were employed.

Compound Distribution and Pharmacokinetics Research:

- The pathways of distribution, metabolism and elimination of epichlorohydrin as a function of dose-route, dose-rate, and dose-frequency in mammals should be investigated.
- Experiments to determine the reaction of epichlorohydrin with various nucleophiles present in biological systems should be conducted to facilitate an understanding of the reaction of epichlorohydrin with cells and their organelles.
- An attempt should be made to correlate any results obtained using in vivo or in vitro testing systems with molecular dosimetry, expressed as either binding to hemoglobin or to DNA in target organs.

2. INTRODUCTION

The 1970 Clean Air Act as amended in 1977 requires that EPA regulate, under Section 112, those pollutants that may reasonably be anticipated to result in an increase in mortality or an increase in serious irreversible, or incapacitating reversible, illness. It also states that EPA must regulate, under Section 111 (d), those pollutants that may reasonably be anticipated to endanger public health or welfare. This health assessment document was requested by the Office of Air Quality Planning and Standards (OAQPS) as a basis for evaluation of epichlorohydrin as a hazardous pollutant. It is envisioned by the Office of Health and Environment Assessment to be one of several information sources to guide regulatory strategies of the OAQPS and other EPA program offices.

In the development of this assessment document, the scientific literature has been inventoried, the studies evaluated, and summary conclusions prepared to identify qualitatively the chemical toxicity and related characteristics. Observed effect levels and other measures of dose-response relationships are discussed. In assessing the health effects of human exposure to epichlorohydrin, few epidemiologic studies were available. The effects in humans have generally been ascertained from either occupational or accidental exposures, and little information has been reported on the concentrations associated with these exposures. Thus, it has been necessary to rely on animal studies to derive indications of potential harmful effects in relation to dose or exposure levels.

Key animal studies are presented in a descriptive manner that includes information on the test organism, dosage regimen and schedule of exposures, duration of exposure, life expectancy of the animal, duration of the experiment, types of effects seen with each dosage, number of test groups and controls, number of animals per group, and sex and age of animals. Statistical significance, coefficients of variation, and the purity of the test material are specified when the data were available. Anecdotal reports are covered in a concise form, but key studies have been expanded for discussion to reach a "weight-of-evidence" summarization within each section.

The major topics included in this document are: physical and chemical properties, sampling and analytical methods, production and use, levels and sources in the environment, fate and transport, and biological effects, including the effects of epichlorohydrin on ecosystems and aquatic species. Biological effects have been defined to include metabolism and pharmacokinetics as well as toxicity to organ and tissue systems, carcinogenicity, mutagenicity, teratogenicity, and reproduction. Human data on the effects of epichlorohydrin are presented and interpreted in terms of data from animal experimentation.

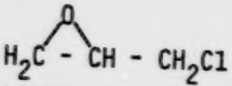
This document is intended to serve as part of the basis for decision-making in the various regulatory offices within the EPA as well as to inform the general public of the nature and extent of information available for assessment of health hazards resulting from environmental exposure to epichlorohydrin.

3. BACKGROUND INFORMATION

3.1 PHYSICAL AND CHEMICAL PROPERTIES

3.1.1 Introduction

Epichlorohydrin is a chlorinated derivative of α, β-propylene oxide and has the formula:



$$H_2\overset{O}{C - CH} - CH_2Cl$$

It is a clear, colorless, highly reactive liquid at ambient temperature and has a chloroform or garlic-like odor. It is both volatile and flammable (Weast 1978). Some of the relevant physical and chemical properties of epichlorohydrin are listed in Table 3-1.

3.1.2. Synonyms and Trade Names

Epichlorohydrin has the following synonyms and trade names:

ECH
ECHH
1-chloro-2,3-epoxypropane
3-chloro-1,2-epoxypropane
(chloromethyl) ethylene oxide
2-(chloromethyl) oxirane
chloropropylene oxide
γ-chloropropylene oxide
3-chloropropene 1,2-oxide
2-chloromethyl oxirane
glycidyl chloride
3-chloro-1,2-propylene oxide
α-epichlorohydrin
(DL)-α-epichlorohydrin
SKEKhG
1,2-epoxy-3-chloropropane
2,3-epoxypropyl chloride
glycerol epichlorohydrine

3.1.3. Identification Numbers

Epichlorohydrin has three commonly used identification numbers:

1. Chemical Abstracts Service (CAS) No. 106-89-8,
2. Registry of Toxic Effects of Chemical Substances (RTECS) No. TX 49000, and
3. U.S.EPA No. A762-1952.

3.1.4. Significance of Physical Properties with Respect to Environmental Behavior

Epichlorohydrin is miscible with ethanol, diethyl ether, acetone, and chlorinated aliphatic hydrocarbons, and slightly soluble in petroleum hydrocarbons

TABLE 3-1. PHYSICAL AND CHEMICAL PROPERTIES OF EPICHLOROHYDRIN

MOLECULAR FORMULA, MOLECULAR WEIGHT, AND ELEMENTAL COMPOSITION

Molecular Formula: C_3H_5OCl
Molecular Weight: 92.53
Elemental Composition:
$C = 38.94\%$
$H = 5.45\%$
$Cl = 38.32\%$
$O = 17.29\%$

PHYSICAL PROPERTIES

Melting Point (Weast 1978)	-48.0° C
Freezing Point (Shell 1969) (Dow 1980)	-57.2° C -57.1° C
Boiling Point (Shell 1969) (Dow 1980)	116.11° C (760 mmHg) 116.07° C (760 mmHg)
Density (g/ml, 20° C) (Shell 1969)	d_4^{20} 1.1812; d_4^{25} 1.1750
Specific Gravity (20/20° C) (Shell 1969)	1.181
Vapor Pressure (16.6° C) (Sax 1975) (30° C) Verschueren 1977)	10 mmHg 22 mmHg
Concentration in Saturated Air (760 mmHg, 25° C) (Hine et al. 1981)	1.7%
Coefficient of Expansion at 68° F (Shell 1969)	0.000577 per °F
Solubility (Shell 1969) Water (10° C) Water (20° C)	 6.52% 6.58%
Pounds per Gallon (68° F) (Shell 1969)	9.58 lbs.
Flash Point (Tag open cup) (Shell 1969) (Tag closed cup) (Dow 1980)	41° C 31° C
Autoignition Temperature (Dow 1980)	416° C
Latent Heat of Vaporization (calc.) (Shell 1969)	9060 cal/mole at the b.p.
Odor Threshold in Air (Hine et al. 1981)	10 ppm
Surface Tension (20° C) (Shell 1969)	37.00 dynes/cm
Heat of Combustion (Shell 1969)	4524.4 cal/gm
Liquid Viscosity (25° C) (Shell 1969)	0.0103 poises
Refractive Index (25° C) (Shell 1969)	n_D1.4358
Heat Capacity (25° C) (Dow 1980) (100° C) (Dow 1980)	31.5 cal/mol° C 40.0 cal/mol° C
Heat of Formation (25° C) (Dow 1980)	-35.6 kcal/mol
Explosive Limits (volume % in Air) (Dow 1980)	3.8-21.0
Heat of Fusion (25° C) (Dow 1980)	2,500 cal/mol

and water. Epichlorohydrin forms an azeotrope with water, distilling at 88° C and containing 75 percent epichlorohydrin by weight (Riesser 1978).

Epichlorohydrin has weak ultraviolet absorption; the exact spectrum is not reported. Based upon its structure, which combines alkyl halide and alkyl epoxide properties, it is reasonable to infer that the maximum absorption will be below 300 nm, the lower cutoff for sunlight due to atmospheric absorption.

Hydrolysis of epichlorohydrin is slow at room temperature but is accelerated by heat or traces of acid or base. Reactions with compounds containing active hydrogen (e.g., alcohols, primary or secondary amines) normally occur initially at the more reactive epoxide site of the molecule, although reactions involving initial displacement of chlorine are also known to occur (Massiot and Levy 1981).

The volatility of epichlorohydrin, as indicated by its relatively high vapor pressure, may lead to transfer from water or soil to the air phase. The details of the environmental fate of epichlorohydrin as determined by its physical and chemical properties are discussed in Section 3.4.

An estimated value of the log octanol/water partition coefficient, using the method of Hansch and Leo (1979) is 0.26 ± 0.04. This indicates a low affinity of epichlorohydrin toward fats or soil. More details are presented in Section 9.2.

3.1.5. Chemical Reactions

Although the epichlorohydrin molecule has two available reactive sites (the chlorine atom and the epoxy group), the epoxy group dominates the reactive character of the compound. The three-membered epoxide ring is highly strained, making its bonds weaker than those of linear ethers. The result is a less stable molecule that will readily undergo acid-catalyzed reactions and cleavage by bases. It is this high degree of reactivity to which epichlorohydrin owes its industrial importance as a chemical intermediate (Dow 1980).

In most of its reactions, epichlorohydrin behaves as an epoxide, initially reacting through the epoxy group with substances containing an active hydrogen atom and with numerous other diverse compounds to form chlorohydrin derivatives. This is true even of its behavior towards substances such as tertiary nitrogen bases, metal alkoxides, and organic acid salts, which will normally effect direct replacement of an active organic halogen atom. The chlorine can be eliminated as hydrogen chloride in a subsequent step involving displacement by the initially-generated, hydroxy group. Glycidol derivatives are thus formed and undergo the addition reactions typical of epoxy compounds (Shell 1969).

By suitable adjustment of reaction conditions, epichlorohydrin can be an intermediate in the synthesis of a wide variety of products (see Shell Chemical Company 1969 for more details). These transformations are illustrated by the reaction of epichlorohydrin with alcohols. In the presence of an acidic catalyst such as stannic chloride, 3-chloro-2-hydroxypropyl ethers are formed in high yields (see reaction 1 in Table 3-2).

Table 3-2 lists the typical reactions that have been observed with epichlorohydrin. A number of these reactions are of commercial importance and are discussed in Section 3.3.

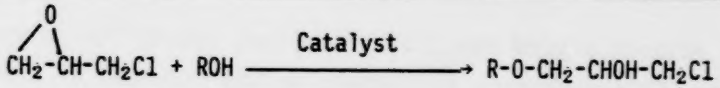
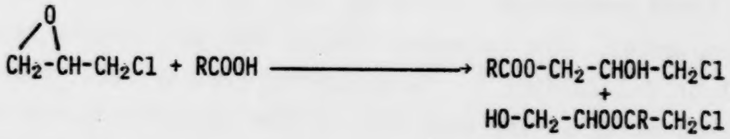
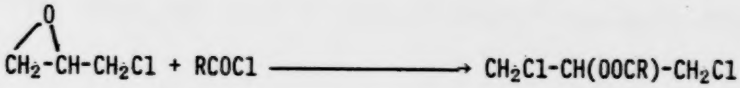
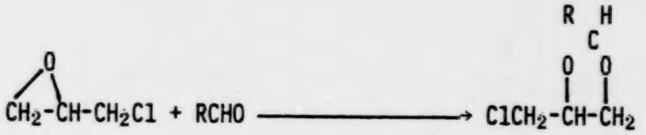
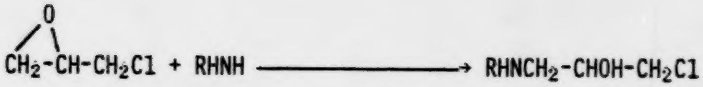
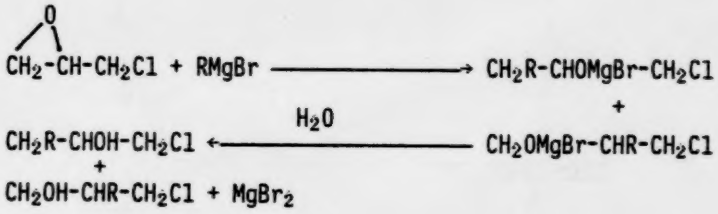
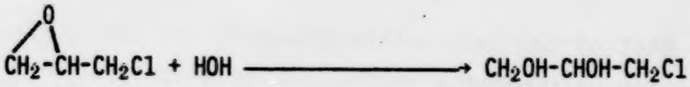
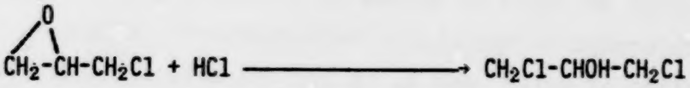
3.1.6 Chemical Reactions in the Environment

Although available literature provides a good description of hydrolysis and related reactions of epichlorohydrin in the laboratory, little information was available on its photochemistry or oxidation in air, water, or soil. Epichlorohydrin is not persistent and appears to hydrolyze in several weeks' time under laboratory conditions (Brönsted et. al. 1929) but reports of field studies on epichlorohydrin were not found in the literature. Removal processes may be possible to predict for epichlorohydrin in air based on molecular structure. These processes would include reaction with hydroxyl radicals, or to a lesser extent with ozone. The estimated rate constant for reaction of epichlorohydrin with the hydroxyl radical is 2×10^{-12} cm^3 $molecule^{-1}$ sec^{-1} (U.S. EPA, 1980). The atmospheric residence time was estimated to be 5.8 days; photolysis was considered to be possible but not probable (see section 3.1.6.3).

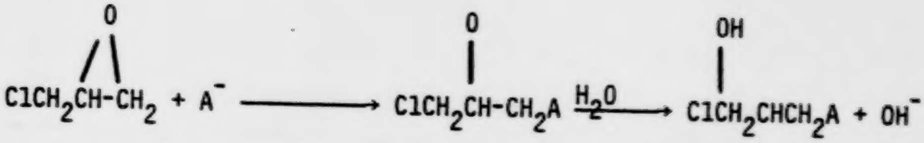
3.1.6.1 Hydrolysis and Related Reactions--Epichlorohydrin hydrolyzes by a complex scheme. Many papers (e.g., Ross 1950; Addy and Parks 1965) delineate mechanisms and rates of epoxide hydrolysis, but none of the studies has investigated hydrolysis under environmental conditions.

The chlorine atom does not react or directly participate in the initial hydrolysis, but it does affect the initial hydrolysis rate by its inductive effects (Ross 1950 and 1962 as cited in NIOSH 1976a; Pritchard and Long 1956; Pritchard and Siddiqui 1973; Kwart and Goodman 1960). Epichlorohydrin can hydrolyze by two general mechanisms: uncatalyzed and acid-catalyzed (Brönsted et al. 1929; Ross 1950). In the uncatalyzed reactions, the rate-determining step involves opening of the epoxide ring by the attack of water, an anion, or other nucleophile at the C-1 carbon (Brönsted et al. 1929; Kwart and Goodman 1960; Long and Pritchard 1956; Addy and Parker 1965):

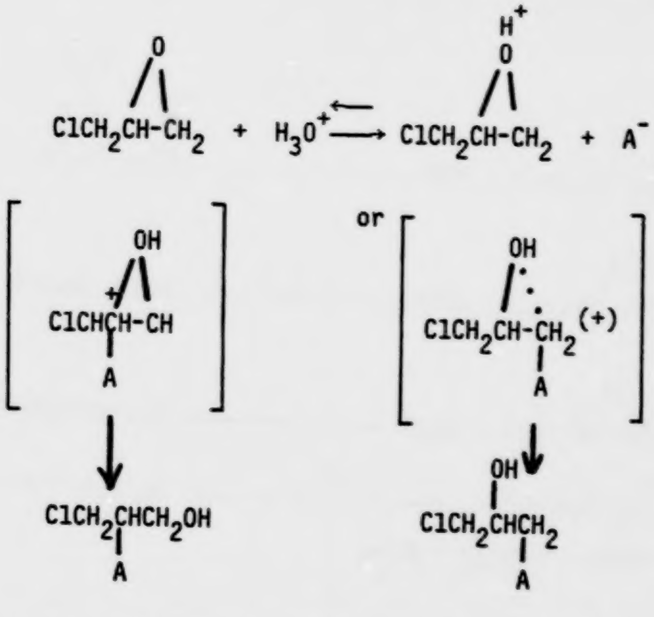
TABLE 3-2. TYPICAL REACTIONS OF EPICHLOROHYDRIN

Monohydric Alcohols	 O CH₂-CH-CH₂Cl + ROH Catalyst R-O-CH₂-CHOH-CH₂Cl
Organic Acids	 O CH₂-CH-CH₂Cl + RCOOH RCOO-CH₂-CHOH-CH₂Cl + HO-CH₂-CHOOCR-CH₂Cl
Acyl Chlorides	 O CH₂-CH-CH₂Cl + RCOCl CH₂Cl-CH(OOCR)-CH₂Cl
Aldehydes	 O CH₂-CH-CH₂Cl + RCHO R H C O O ClCH₂-CH-CH₂
Amines	 O CH₂-CH-CH₂Cl + RHNH RHNCH₂-CHOH-CH₂Cl
Grignard Reagents	 O CH₂-CH-CH₂Cl + RMgBr CH₂R-CHOMgBr-CH₂Cl + CH₂OMgBr-CHR-CH₂Cl H₂O CH₂R-CHOH-CH₂Cl + CH₂OH-CHR-CH₂Cl + MgBr₂
Water	 O CH₂-CH-CH₂Cl + HOH CH₂OH-CHOH-CH₂Cl
Inorganic Acids	 O CH₂-CH-CH₂Cl + HCl CH₂Cl-CHOH-CH₂Cl

Source: DOW (1980)



The acid-catalyzed reactions have been identified as having an A-2 type mechanism. This mechanism may depend on the strength of the acid. Epichlorohydrin is first protonated reversibly, and the protonated compound reacts with water or an anion. The rate-determining step can involve ring opening at either the C-1 or C-2 carbon; in the acid-catalyzed process, opening at C-2 may be preferred owing to the stability of the secondary carbonium ion (Brönsted et al. 1929; Long and Pritchard 1956; le Noble and Duffy 1964):



Most of the information necessary for the product and half-life calculations for environmental hydrolysis of epichlorohydrin either was experimentally measured or could be estimated from available data. Table 3-3 summarizes the epichlorohydrin hydrolysis rate constants, k_1 and k_2 (k_1 is for the uncatalyzed addition of water, and k_2 is for the acid-catalyzed addition of water). Table 3-4 lists the experimentally derived rate constants, k_3 and k_4, for anion reactions with epichlorohydrin (k_3 is for the uncatalyzed addition of an anion, and k_4 is the acid-catalyzed addition of an anion).

Table 3-3. Rate Constants for Hydrolysis of Epichlorohydrin as a Function of Temperature[a]

Temperature (°C)	$10^6 k_1$[b] (s^{-1})	$10^5 k_2$[c] (liter $mol^{-1} s^{-1}$)	Reference
0.0		6.91	Pritchard and Siddiqui (1973)
20.0	0.97	43.4	Pritchard and Siddiqui (1973)
25.0		68.2, 77	Brönsted et al. (1929) le Noble and Duffy (1964) Pritchard and Siddiqui (1973)
35.0	5.9		Shvets and Aleksanyan (1973)
37.0	5.3		Ross (1962 as cited in NIOSH 1976a)
45.0	13.8		Shvets and Aleksanyan (1973)
50.0	20.4		Shvets and Aleksanyan (1973)
75.0	129		Shvets and Aleksanyan (1973)
85.0	246		Shvets and Aleksanyan (1973)

[a] Reaction conditions are indicated in each reference.

[b] Rate for uncatalyzed addition of water.

[c] Rate for acid-catalyzed addition of water.

Table 3-4. Rate Constants for Epichlorohydrin Reaction with Various Anions

Anion	°C	$10^5 k_3$[a] (liter mol^{-1}s^{-1})	$10^2 k_4$[b] (liter^2 mol^{-2}s^{-1})
Chloride, Cl^-	20	1.15[c], 0.99[d]	0.45[c]
	40	6.3[e]	6.8[e]
Iodide, I^-	20	10.0[b]	
Thiosulfate, $S_2O_3^{-2}$	20	6.3[b]	
Formate, HCO_2^-	20	0.47[b]	
Benzoate, $C_6H_5CO_2^-$	20	0.52[b]	
Acetate, $CH_3CO_2^-$	20	0.62[b]	
	37	3.33[c]	
Nitrate, NO_3^-	20	0.022[f]	
Bicarbonate, HCO_3^-	65	0.17[g]	
	75	0.30[g]	
	80	0.52[g]	
	85	0.68[g]	
Carbonate, CO_3^{-2}	35	0.42[g]	
	45	0.83[g]	
	50	1.42[g]	
	60	2.5[g]	

[a] Rate for uncatalyzed addition of anion.

[b] Rate for acid-catalyzed addition of anion.

[c] Brönsted et al. (1929).

[d] Ross (1950)

[e] Addy and Parker (1965).

[f] Petty and Nichols (1954).

[g] Shvets and Aleksanyan (1973).

The hydrolytic half-life for epichlorohydrin at 20°C in distilled water was determined to be 8 days (Brönsted et al. 1929). Ross (1950) reported the rates of first-order hydrolytic reactions of epichlorohydrin at 37°C under acidic and neutral conditions (Table 3-5).

Epichlorohydrin is expected to hydrolyze faster if the water has a high chloride or high carbonate-bicarbonate content. Although the hydrolysis product is 3-chloro-1,2-propanediol, significant concentrations of other products, including 1,3-dichloro-2-propanol, can be formed from further reactions with aqueous anions.

Table 3-5. Rate of Reaction of Epichlorohydrin (ECH) under Neutral and Buffered Conditions at 37°C

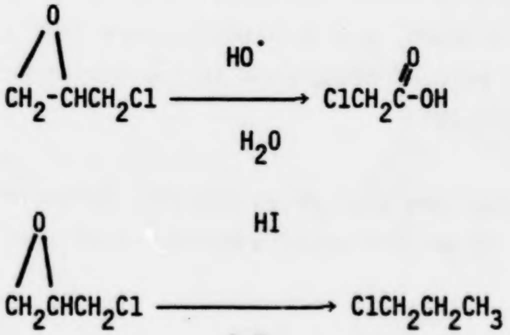
Duration of Reaction (h)	Neutral Condition[a] % ECH Reacting	Neutral Condition[a] k_1 (h^{-1})	Buffered Condition[b] % ECH Reacting	Buffered Condition[b] k_1 (h^{-1})
24	36.5	0.0190	52.0	0.0185
48	59.0	0.0185	76.6	0.0185
72	72.5	0.0180	88.0	0.0180
100	84.5	0.0185	96.0	0.0185
169	96.5	0.0200	100.0	--

[a] Relates to reaction in water.

[b] Relates to reaction in water containing 0.1 M sodium acetate and 0.1 N acetic acid.

Source: Ross (1950).

3.1.6.2 Oxidation -- Shell (1969) lists several oxidation and reduction reactions of epichlorohydrin, none of which is environmentally significant, for example:



Epichlorohydrin can be oxidized by free radical processes in liquid (Dobbs et al. 1976; Beckwith 1982) or gas phases (Dilling et al. 1976); these reactions may occur as photochemically initiated atmospheric reactions (Gay and Bufalini; 1971; Bufalini 1971). The liquid phase, free radical oxidations discussed here are probably not important in the environment, but are possible mechanisms by which epichlorohydrin could be oxidized by atmospheric free radical initiators.

Available literature evaluates the mechanisms of liquid phase reactions with a few free radical initiators. The structure of the free radical produced from epichlorohydrin depends in part upon the radical initiator. Dobbs et al. (1976) suggested that the t-butoxyl radical, $(CH_3)_3CO$, preferentially abstracts a hydrogen atom from an alicyclic carbon, whereas the hydroxyl radical, HO, preferentially abstracts one from an acyclic carbon. Their experimental work with epichlorohydrin was limited to the identification of species formed by reactions with hydroxyl radicals produced by the titanium (III) ion-hydrogen peroxide system.

3.1.6.3 Photolysis -- No ultraviolet absorption data were available for epichlorohydrin. Neither the alkyl halide nor the epoxide portion are expected to have strong absorption in the sunlight region (wavelengths above 300 nm) (Calvert and Pitts 1966). Epichlorohydrin's absorption maximum is probably below 250 nm and, at most, is expected to have only minimal absorption above 300 nm. No significant direct photochemical reactions are expected under environmental conditions. Indirect processes involving reactions other than photochemically generated species are of course possible.

No reports on the photodegradation of epichlorohydrin in the environment were found in the literature. In a laboratory study, Dilling et al. (1976) determined the decomposition rate at 27 ± 1° C of 10 ppm epichlorohydrin in an atmosphere containing 5 ppm nitric oxide (NO). Two 275-W reflector sunlamps (which had a short wavelength cutoff of 290 nm) were used as UV sources. The intensity was stated to be about 2.6 times that of natural sunlight at noon on a summer day in Freeport, Texas. The rate of disappearance of epichlorohydrin was determined by a gas chromatograph with a flame ionization detector. The half-life reported from these studies was 16.0 hours, and no products were identified. Extrapolation from these results to the rate of photolysis in the environment is not justified.

3.2 ANALYTICAL METHODOLOGY

3.2.1 Introduction

Several approaches have been developed for determining the quantity of epichlorohydrin present in environmental samples. Four useful analytical methods that have been described include: (1) volumetric determination (Swan 1954); (2) oxidation and colorimetric determination at 412 nm (Jaraczenska and Kaszper 1967); (3) extraction with carbon tetrachloride and spectrophotometric determination of absorbence at 1,274 cm^{-1} (Adamek and Peterka 1971); and (4) ring opening by halogen acids and quantification of the hydrogen halide (Dobinson et al. 1969).

There are several other methods that have been developed for epichlorohydrin and other epoxides (Dobinson et al. 1969) that may have specific applications. These methods vary widely in specificity and sensitivity. Methods for analysis of air and water are described below.

3.2.2 Chemical Analysis in Air

Several methods exist for measuring epichlorohydrin concentrations in air. Daniel and Gage (1956) described a sensitive colorimetric method for measuring epichlorohydrin vapor. This method is based on the oxidation of epichlorohydrin with periodic acid, followed by reaction of the formaldehyde with ammonia and acetylacetone to give a yellow-colored solution. The method is capable of giving a reasonably accurate result with as little as 20 ug of epichlorohydrin and is therefore capable of analyzing atmospheric concentrations of 10 mg/cm^3 using a 2-liter sample. The analytical error is estimated at about 2 percent.

The analysis of epichlorohydrin in workroom air is best achieved using an adsorption technique and gas chromatography. NIOSH (1976a) recommended sampling using activated charcoal as the adsorbent. The determination of epichlorohydrin at the level of a few parts per billion has been performed using gas chromatography-mass spectrometry, which provides the highest sensitivity with high specificity (van Lierop 1978).

A standard sampling and analytical method for epichlorohydrin has been developed by NIOSH (1976a). The method involves trapping epichlorohydrin vapor from a known volume of air on charcoal and then desorbing it with carbon disulfide. An aliquot of the desorbed sample is injected into a gas chromatograph; the area of the resulting peak is determined and compared with standards. This method was developed to analyze epichlorohydrin over the range of 11.7 to 43.1 mg/m^3 at an atmospheric temperature of 23° C and a pressure of 765 mmHg. For a 20-liter sample, the useful range of this method is 2 to 60 mg/m^3 at a detector sensitivity that gives nearly full deflection on a strip chart recorder for a 1 mg sample. The method is capable of measuring levels as low as 50 ppb

(NIOSH 1976a). Any compound that has about the same retention time as epichlorohydrin under the gas-chromatographic conditions used in this method will interfere with the analysis.

A portable, battery-operated gas analyzer and a detector tube are available from at least one instrument supplier for the detection of epichlorohydrin in air (AIHA 1961).

Anderson et al. (1981) reported the results of a comparison of activated charcoal, Amberlite XAD-2, and Amberlite XAD-7 for sampling of epichlorohydrin in workroom air. Amberlite XAD-7 was observed to be an excellent adsorbent for epichlorohydrin, giving high recoveries and no decomposition. Percent recovery of 8, 40, and 400 μg samples with a dichloromethane eluent was between 99 and 100 percent, with a range of estimated standard deviations between 1.2 and 1.7.

3.2.3 Chemical Analysis in Water

Some of the methods used for measuring epichlorohydrin levels in water are essentially the same as those used for levels in air. Many of the methods for analysis in air involve first trapping the epichlorohydrin in an aqueous medium.

Daniel and Gage (1956) described a sensitive colorimetric method for determining levels of epichlorohydrin in water based on oxidation with periodic acid to form formaldehyde and then reaction with ammonia and acetylacetone to form 3,5-diacetyl-1,4-dehydrolutidine, which has a yellow color. The color is allowed to develop, and the optical density of the sample is measured with a spectrophotometer at 412 mm. This method is accurate to 20 μg in a 15 ml water sample (1.3 ppm).

In another method (Dobinson et al. 1969), aqueous samples at concentrations ranging between 10 and 1,000 ppm may be analyzed by treatment with alcoholic potassium hydroxide and then determining the generated chloride ions potentiometrically using aqueous silver nitrate solution.

Epichlorohydrin may also be determined in water samples by extracting the samples with carbon tetrachloride (Adamek and Peterka 1971). The carbon tetrachloride is then analyzed for epichlorohydrin using infrared spectrophotometry. The analytical band used for quantification is at 1,274 cm^{-1}, using a 1-mm cell thickness and a 10-ml water sample. The limit of quantification was approximately 0.03 percent (300 ppm). Infrared spectroscopy is a convenient and rapid analytical technique; however, in this assay, the sensitivity was low. It may be increased by extracting larger water samples and using a greater cell thickness.

3.3 PRODUCTION, USE, AND RELEASES TO THE ENVIRONMENT

3.3.1 Introduction

The purpose of this document is to present available information relevant to human health effects of epichlorohydrin. Available information regarding sources, emissions, and ambient air concentrations, has been included only to give the reader a preliminary indication of the potential presence of this substance in the ambient air. While the available information is presented as accurately as possible, this discussion is acknowledged to be based on limited data and is not intended to be used alone to make regulatory conclusions regarding risks to public health.

If a review of the health information indicates that the Agency should consider regulatory action for epichlorohydrin, a considerable effort will be undertaken to obtain more extensive information regarding sources, emissions, and ambient air concentrations. Such additional data will provide information for drawing regulatory conclusions regarding the extent and significance of public exposure to epichlorohydrin.

3.3.2 Production

Epichlorohydrin is produced commercially in the U.S. by the chlorination process. The chlorination process is a three-step series of reactions. The first step is the production of allyl chloride from propylene and chlorine. The allyl chloride is used to provide epichlorohydrin by hypochlorination and subsequent neutralization. Currently, all the allyl chloride produced in the U.S. is used in the production of epichlorohydrin. Crude epichlorohydrin may be transferred directly to the glycerine production step. Refined epichlorohydrin is sold for other uses (Blackford, 1978).

In the U.S., production of epichlorohydrin started in about 1937 and expanded in 1949 in connection with the first synthetic glycerine plant. Epichlorohydrin is currently produced by two companies, Shell Chemical Company (Deer Park, Texas; Norco, Louisiana) and Dow Chemical U.S.A. (Freeport, Texas) by the chlorohydrination of allyl chloride. Ciba-Geigy Corp. (Toms River, New Jersey) and Union Carbide Corp. (South Charleston, West Virginia) have in the past produced, or have the capacity to produce, epichlorohydrin. Production of crude epichlorohydrin by Shell and Dow in 1977 was about 296 million pounds (135 million kg). Estimated production of refined epichlorohydrin by Shell and Dow in 1977 was 203 million pounds (92 million kg).

The production of epichlorohydrin for the years 1978 through 1980, based on estimates of the Chemical Information Service (U.S. EPA 1983) are shown in Table 3-6. Estimated capacity for 1982 was 640 million pounds (Shell: 220 million pounds, Dow: 420 million pounds).

3.3.3 USE

The estimated U.S. consumption of epichlorohydrin in 1977 was as follows: synthetic glycerine, 25 percent; unmodified epoxy resins, 53 percent; epichlorohydrin elastomers, 2 percent; other products, 15 percent; and exports, 5 percent. Uses included in the 15 percent consumption for other products are glycidol ethers, some modified epoxy resins, wet strength resins for paper, water treatment resins, surfactants, and ion exchange resins. Domestic consumption data for 1977 are summarized in Table 3-7. In 1978, the United States accounted for 49 percent of the world's total epichlorohydrin use (Blackford 1978).

Table 3-6 Estimation of Epichlorohydrin Production, 1978-1980, in Millions of Pounds.

	1978	1979	1980
Glycerine Feed	60	49	-
Refined Feed	265	310	-
Total	325	350	300

Source: U.S. EPA 1983

Table 3-7 Domestic Consumption of Epichlorohydrin for 1977

Use	million lbs	(million kg)
Crude epichlorohydrin	291	(132)
For synthetic glycerine	75	(34)
For refined epichlorohydrin	216	(98)
Refined Epichlorohydrin	203	(92)
For unmodified epoxy resins	152	(69)
For epichlorohydrin elastomers	7	(3)
For miscellaneous	44	(20)

Source: Blackford 1978

3.3.3.1 Synthetic Glycerine--1977 132 million pounds (60 million kg) of synthetic glycerine were produced. Of that, 66 million pounds (30 million kg) were produced from epichlorohydrin. Approximately 25 percent of crude epichlorohydrin production in 1981 was estimated to be used to produce glycerine (U.S. EPA 1983). About 65.5 percent percent of the synthetic glycerine produced is expected to be derived from epichlorohydrin in 1982 (Blackford 1978).

Glycerine is produced from epichlorohydrin by one company in the U.S., Dow Chemical, Freeport, Texas (SRI 1982). The capacity of Dow's Freeport plant is 115 million pounds (52 million kg) per year. (Chemical Marketing Reporter 1981).

3.3.3.2 Epoxy Resins--The principal application of epichlorohydrin is in the manufacture of epoxy resins. The term "epoxy resin" is assigned to polymeric materials containing epoxide groups. Epoxy resins are commercially used in protective coatings, bondings, adhesives, reinforced plastics, and other products. The consumption of unmodified epoxy resins in the U.S. in 1980 was 317 million lbs. (144 million kgs.)

About 90 percent of commercially produced epoxy resins are made by reaction of epichlorohydrin with 2,2-di(4-hydroxyphenyl) propane. There are many industrial users of epoxy resins; 25 or more generally use epoxy resins as starting materials for their products (Osterhof 1981).

3.3.3.3 Textiles--Epichlorohydrin has been used to esterify the carboxyl groups of wool. The resulting product has both increased life and improved resistance to moths. Epichlorohydrin has been used to prepare protein-modified, wool-like fiber, which has an affinity for acid-dyes and exhibits resistance to both molds and insects. In addition, epichlorohydrin has been used in the preparation of polyacrylonitrile, polyvinyl chloride, polyvinyl alcohol, and other fibers. It has also been used to impart wrinkle resistance and to prepare antistatic agents and textile sizings, and derivatives of epichlorohydrin have shown utility as leveling, dispersing, softening, emulsifying, and washing agents (Dow 1980).

3.3.3.4 Paper, Inks, and Dyes--Wet-strength paper sizing may be prepared from either polyamides modified with epichlorohydrin or from the reaction product of epichlorohydrin and an alkylene amine. In the paper industry, epichlorohydrin adducts are also useful as filler retention aids, paper coatings, flocculants, and antistatic agents. Paper and paperboard products with improved printability, pigment retention, folding endurance, and gloss also have been prepared with epichlorohydrin reaction products (Dow 1980).

Epichlorohydrin polyhydroxy compounds and their esters are useful in the production of special printing inks and textile print pastes. These products yield flexible films that are chemically inert to sodium hydroxide and other chemical solutions (Dow 1980).

3.3.3.5 Anion Exchange Resins--Water-insoluble, anion-exchange resins having good stability may be prepared by reacting epichlorohydrin with ethylenediamine or a high molecular weight homolog. Strong-base, anion-exchange resins can be produced by reacting epichlorohydrin with polymeric tertiary amines. Epichlorohydrin-based anion exchangers have been used successfully to clean polluted air and water. Cation-exchange resins may be produced by the condensation of epichlorohydrin with polyhydroxy phenols followed by sulfonation of the product (Dow 1980).

3.3.3.6 Solvents--Epichlorohydrin is a good solvent for cellulose acetate, rosin, and ester gum (Dow 1980). The reaction of epichlorohydrin with alcohols, alcoholates, and the sodium salts of stearic, oleic, palmitic, myristic, and other fatty acids yields products used as vinyl polymer plasticizers, solvents for food and tobacco flavorings, and as plasticizers for polyurethanes (Dow 1980).

3.3.3.7 Surface Active Agents--A number of epichlorohydrin-based, surface-active agents have been synthesized by condensing epichlorohydrin with a polyamine such as tetraethylene-pentamine plus a fatty acid such as stearic acid. A sulfonated epichlorohydrin derivative has occasionally been substituted for epichlorohydrin. Such products have been found useful in cosmetics and shampoos, and as detergents, sudsing agents, water softeners, and demulsifiers (Dow 1980).

3.3.3.8 Epichlorohydrin-Based Rubber Elastomers--Copolymers of epichlorohydrin with ethylene oxide are members of a new family of specialty polyether rubbers. These elastomers possess desirable properties over a wide range of temperatures and are resistant to gasoline, oil, and ozone. Other advantages are "good aging properties," high resiliency, and flexibility at low temperatures (Dow 1980).

Only about 3 percent of refined epicholorhydrin consumption in the United States in 1982 is expected to be used for the production of epichlorohydrin elastomers. It is estimated that the consumption of epichlorohydrin in manufacturing the elastomers was about 7 million pounds (3.2 million kg) in 1977 (Blackford 1978).

Applications for epichlorohydrin-based rubber include automotive and aircraft parts, seals, gaskets, wire and cable jackets, adhesives, packings, hose and belting, rubber-coated fabrics, and energy-absorbing units (Dow 1980).

3.3.3.9 Starch Modifier--Food starch may be modified by epichlorohydrin to produce stable canned food products. According to Rutledge and Islam (1973), treating rice with epichlorohydrin cross-links the starch granules and produces a stable rice which retains favorable properties after canning.

The U.S. Food and Drug Administration permits food starch to be treated with epichlorohydrin alone and with combinations of epichlorohydrin and propylene oxide, acetic anhydride, and succinic anhydride. Provision has also been made for sequential treatment of starch with epichlorohydrin followed by propylene oxide. The use of these reagents is subject to limitations concerning maximum concentrations of the treatment reagents and maximum allowable concentrations of chemical residues in the treated food. (21 CFR 172.892). Food starch may be treated with epichlorohydrin not to exceed 0.3 percent and propylene oxide not to exceed 10 per cent, residual propylene chlorohydrin in the modified starch not to exceed 5 ppm.

3.3.3.10 Other Current Uses--A variety of other products are produced from epichlorohydrin, most of them in relatively small volumes. Among them are glycidyl ethers, some types of modified epoxy resins, intermediates for plasticizers, dyestuffs, pharmaceuticals, oil emulsifiers, and lubricants (Riesser 1978). It is also used as a stabilizer in chlorine-containing materials such as chlorinated rubber and chlorinated insecticides (Shell 1969; Abdel Sayed et al. 1974).

3.3.3.11 Proposed Uses--Epichlorohydrin has been recommended as a good solvent for cellulose acetate, rosin, and ester gums (Shell 1969), although its toxicity may preclude such use. Dow (1980) has recommended the following additional possible applications of epichlorohydrin or its derived products:

- asphalt improvers
- corrosion inhibitors
- electrical insulation for wire

- filament sizing
- fire-retardant urethanes
- liners for polyethylene bottles
- linoleum and linoleum cements
- lubricant additives
- petroleum production aids
- photographic film bases
- rubber latex coagulation aids
- waterproofing compounds
- zinc electroplating compounds

Epichlorohydrin in conjunction with copper ions has been proposed as a possible spermicidal agent by Kalla and Bansal, 1977. The safety of this combination as a contraceptive agent would have to be demonstrated before this proposed use could be considered seriously.

3.3.4 Substitute Chemicals/Processes

Glycerine has been manufactured by at least three processes other than epichlorohydrin hydrolysis; none require chlorinated hydrocarbons as intermediates. Furthermore, the use of epichlorohydrin in glycerine production has diminished (Blackford 1978).

The unique properties of epoxy resins and epichlorohydrin elastomers are difficult to replace, especially if the use of closely related chemicals such as epibromohydrin or halogenated 1,2-epoxybutanes are also prohibited. Other compounds containing an epoxy ring could be used to make epoxy resins, but the properties of the resins, as well as manufacturing costs, might be adversely affected by substituting them for epichlorohydrin.

3.3.5 Environmental Release

Epichlorohydrin may be released into the environment as a result of its manufacture, use, storage, transport, and disposal. It has been estimated that epichlorohydrin emissions to the atmosphere from the three major production facilities in the United States totaled about 1.47×10^5 pounds (6.7×10^4 kg) in 1978 (Anderson et al. 1980). Releases from these facilities occurred mainly through condenser vents of the distillation columns, although smaller amounts of emissions also came from storage tanks and loading and handling facilities and from plant equipment leaks.

Epichlorohydrin is also released during its use in the production of epoxy resins, elastomers, and miscellaneous products (Anderson, et al. 1980). During 1978, epichlorohydrin was released in at least 11 locations in the

United States during the production of epoxy resins; these emissions totaled about 2.5 x 10^5 pounds/year (1.1 x 10^5 kg/year). An additional 8.1 x 10^4 pounds/year (3.7 x 10^4 kg/year) of epichlorohydrin was estimated to be released during its use in the production of chemicals other than glycerine (Anderson, et al. 1980).

Epichlorohydrin may also be released as a component of industrial effluents and other wastes. No information was found concerning actual modes of waste disposal in the United States.

Epichlorohydrin has been reported to have been released in accidental spills on at least two occasions. In a train accident in January 1963, about 5,000 gallons of epichlorohydrin was spilled into the New River at South Fayette, West Virginia (Gillenwater 1965). In another train accident in January 1978, (also in West Virginia), more than 20,000 gallons of epichlorohydrin were spilled near the center of the town of Point Pleasant, about 150 feet from the Ohio River (Chemical Week 1978). Apparently only in the second case was a cleanup attempted. The chemical was reported not to have contaminated the Ohio River. Local officials ordered the removal of about 1 acre of soil (to several feet deep). The soil was eventually removed to a Dow Chemical Company facility in Texas. The level of epichlorohydrin in water from wells closest to the spill area at the time was 75 ppm. After estimation of the rate of subsurface movement, the city's wells were closed and since then water has been obtained from a radial collector several miles from the city (EPA, 1978).

No empirical information on epichlorohydrin release rates from landfills or lagoons was found. In a theoretical discussion, Falco, et al. (1980) developed a model to predict the transport, sorption, and degradation properties of epichlorohydrin and other organic chemicals from waste disposal sites. This predictive model indicated that sorption of epichlorohydrin onto soil from groundwater is unlikely, and that "approximately 100%" of the compound released from unconfined landfills and lagoons would reach surface waters. Additional results of this model are discussed in Section 3.4.1.2.2.

3.3.6 Environmental Occurrence

Hushon, et al. (1980) indicated that epichlorohydrin had been identified in water samples from an oil refinery, in industrial effluents, and in surface water. However, no sample concentrations were reported and no information was provided concerning the location from which the samples were collected. Levels of epichlorohydrin in the ambient air have not been determined.

3.4 ENVIRONMENTAL TRANSPORT AND FATE

The ultimate environmental fate of epichlorohydrin depends on its release, transport, and persistance characteristics. Epichlorohydrin is known to be released into (1) the atmospheric compartment as a result of manufacture and use, (2) the aquatic compartment with industrial effluents, and (3) the terrestrial compartment due to spills and dumping. Upon release, epichlorohydrin is not expected to persist. If released at the water/soil interface, epichlorohydrin's water solubility, estimated soil adsorption coefficient, and predicted behavior when released from a landfill indicate the compound will move into the aquatic compartment. Should epichlorohydrin be released at the air/soil interface, high volatility of the compound and soil mobility suggest it will favor the atmosphere. At the air/water interface, the volatility, solubility, and other data indicate epichlorohydrin will partition into both media. In the discussion below, the environmental fate of epichlorohydrin is assessed in light of its transport and persistence properties.

3.4.1 Transport

3.4.1.1 Volatilization--Epichlorohydrin is a volatile liquid with a latent heat of vaporization of 9,060 cal/mole at the boiling point (Hawley 1977; Riesser 1978). Based on its vapor pressure, reported to be 10 mmHg at 16.6° C (Sax 1975) and 22 mmHg at 30° C (Verschueren 1977), it is expected to volatilize under normal environmental conditions. Although its evaporation half-life has not been experimentally determined, it may be predicted using the model system proposed by Dilling et al. (1976) and Dilling (1977). Based on a 1 mg/l aqueous solution in a total volume of 250 ml or water, 6.5 cm deep and stirred at 200 rpm at 20° C, the predicted evaporation half-life of epichlorohydrin is 0.15 days (2.37 days if the depth is 100 cm). The calculations are shown in Appendix A. These experimental conditions will not be encountered in natural aquatic environments; therefore, the actual half-life in the environment may differ from these data.

3.4.1.2 Sorption

3.4.1.2.1 Soils. The soil-water partition coefficient per unit organic matter (K_{oc}) for epichlorohydrin was estimated using the regression equation of Kenaga and Goring (1980): $\log K_{oc} = 3.64-0.55$ (log water solubility) ± 1.23 orders of magnitude (see Appendix B). The calculated K_{oc} values range from $10.28 \times 10^{-1.23}$ (0.61) to $10.28 \times 10^{1.23}$ (174) at a solubility of 60,000 mg/l and 9.76 x $10^{-1.23}$ (0.57) to 9.76 x $10^{1.23}$ (166) at 66,000 mg/l. Although actual experimental determination of K_{oc} could yield a value different from those estimated, epichlorohydrin does have a low estimated K_{oc} (i.e., 100). Thus, it would have a low potential for soil adsorption.

An alternative method of estimation developed by Briggs (1973) indicates epichlorohydrin to be "mobile" in soils. The soil organic matter/water partition coefficient, Q, was calculated (see Appendix B) by the equation:

$$\log Q = 0.524 (\log P) + 0.618$$

where P is the octanol/water partition coefficient. Log P was estimated according to the method of Hansch and Leo (1979) (see Appendix C). The calculated Q value of 5.68 indicates the compound will be "mobile" in soils, according to Briggs' (1973) rating system, and prefer the aqueous medium to soil.

3.4.1.2.2 Sediments. Epichlorohydrin, with its estimated K_{oc} of 100, is predicted to have a low potential for sorption onto stream sediments (Kenaga and Goring 1980). Using another mathematical approach, the EXAMS Model, Falco et al. (1980) estimated the transport, sorption, and degradation properties of various organic chemicals from waste disposal sites. The model's results for epichlorohydrin indicated that sorption onto soil and sediment particles would not be important processes. The authors stated that approximately 100 percent of the compound released from unconfined landfills and lagoons would reach surface waters. Falco et al. (1980) also reported that the potential for contamination of bottom sediments in water bodies would be low because the model predicted that concentrations in the sediments of a river, a pond, and a lake would each be only 20 percent of the overlying water concentrations. Sorption of epichlorohydrin onto sediments suspended within the water column would also be very low; it was predicted that only 0.001 percent would be sorbed onto suspended sediments in a river reach traversed in 5 days (50-250 miles), 0.01 percent onto suspended sediments in a pond with a 100-day retention time, and 0.001 percent onto suspended sediments in a reservoir or lake with a 365-day retention time (Falco et al. 1980).

3.4.2 Fate

3.4.2.1 Chemical and Physical Process--The major chemical processes affecting the environmental fate of epichlorohydrin are hydrolysis and oxidation. These are discussed in more detail in Sections 3.1.6.1 and 3.1.6.2.

Physical processes which may remove epichlorohydrin from the atmosphere include adsorption on aerosol particles and subsequent removal, or adsorption by soil and water at the earth's surface. Photodegradation is not expected to be a significant potential environmental process. It is discussed in Section 3.1.6.3.

3.4.2.2 Biological Processes--Experimental data on bioaccumulation of epichlorohydrin were not found in the literature. The aqueous reactivity, log P value, and hydrolytic properties of epichlorohydrin suggest there is a low potential for bioaccumulation or bioconcentration in aquatic and terrestrial food chains. Published bioconcentration factors are based on either water solubility or octanol/ water partition coefficients. Four different equations for estimating bioconcentration factors (BCF) are presented in Appendix D. Two equations based on water solubility are those of Chiou et al. (1977) and Lu and Metcalf (1975). Two other equations based on log P values were reported by Neely et al. (1974) and Veith et al. (1980). The log BCF values for epichlorohydrin calculated by these four equations range from -0.032 to 0.968. Log BCF values of less than 2 indicate low bioconcentration potential (Kenaga 1980).

Information on microbial biodegradation of epichlorohydrin is limited. Epichlorohydrin was identified as an intermediate in the enzymatic hydrolysis of 2,3-dibromo-1-propanol when chloride ions were present (Castro and Bartinicki 1968). Unspecified cultures of Flavobacterium were isolated from soil in an alfalfa field and grown in a medium containing 0.005 M dibromopropanol. A crude enzyme extract was obtained from the bacteria by centrifugation of sonicated cells. The activity of the crude extract was reported to be equivalent to the activity of the cell suspension. The crude enzyme extract was partially purified, precipitated, and then passed through a Sephadex G-200 column. Dibromopropanol, epichlorohydrin, and epibromohydrin were each metabolized by both the crude enzyme extract and the purified extract at pH 7. Castro and Bartinicki (1968) observed that 2,3-dibromo-1-propanol was first converted into epibromohydrin. The next step depended on the presence of bromide or chloride ions. If bromide ions were present, the epoxide group opened to yield 1,3-dibromopropanol. However, if chloride ions were present, epibromohydrin was converted to epichlorohydrin.

The biochemical degradation of epichlorohydrin was studied by Bridié et al. (1979a) using unidentified seed cultures. They reported the theoretical oxygen demand (TOD), biochemical oxygen demand (BOD), and the chemical oxygen demand (COD). Five-day BOD's were measured using the American Public Health Association's Standard Method No. 219 published in 1971. The method was modified by adding 0.5 mg/l allylthiourea to prevent nitrification. The seed cultures were obtained from a biological sanitary waste treatment plant, and duplicates were checked for activity using a mixture of glucose and glutamic acid. COD was obtained by the standard potassium dichromate method ASTM D 1252-67 published by the American Society for Testing and Materials in 1974. The BOD's for epichlorohydrin using unadapted and adapted seed culture were 3 and 14 percent of the TOD, respectively. In comparison, the BOD's with unadapted seed were 82 percent for glycerine and 1 percent for dichloropropanol (Bridié et al. 1979a).

3.5 SUMMARY

In 1980, 300 million pounds (136 million kg) of epichlorohydrin were produced in the U.S. by Shell Chemical Company and Dow Chemical U.S.A. (U.S. EPA 1983). Epichlorohydrin is used to produce epoxy resins, synthetic glycerine, elastomers, and other products.

Emissions of epichlorohydrin to the atmosphere from production in 1978 were estimated to be 1.47 x 10^5 pounds (6.7 x 10^4 kg). Emissions from epoxy resin production in 1978 were estimated to be 2.5 x 10^5 pounds (1.1 x 10^5 kg). An additional 8.1 x 10^4 pounds (3.7 x 10^4 kg) of epichlorohydrin were estimated to be released during its use in the production of chemicals other than glycerine (Anderson 1980). No data were found regarding release rates into water bodies, landfills, or lagoons; although several accidental spills have been documented. Levels of epichlorohydrin present in the atmosphere were not available.

Epichlorohydrin released into the environment is not expected to persist. Its behavior relative to air, soil, and water have been assessed based on solubility, volatization, and other properties. It is predicted to have low potential for soil and sediment adsorption, and that 100 percent of epichlorohydrin released from a lagoon or landfill would reach surface waters. Epichlorohydrin released to the atmosphere would be removed by chemical and physical processes; its atmospheric half-life is calculated to be 5.8 days.

There is low potential for bioaccumulation of epichlorohydrin. Little is known about the biodegradation of epichlorohydrin.

4. COMPOUND DISTRIBUTION AND RELATED PHARMACOKINETICS

The major route of exposure to epichlorohydrin in humans is through the respiratory tract. There is also potential for dermal exposure. Exposure via the oral route is expected to be slight; however, the possibility exists that exposure by this route could occur as a result of ingesting contaminated water or the leaching or unpolymerized epichlorohydrin from plastic wrap or plastic containers into food.

4.1 ROUTES OF EXPOSURE AND ABSORPTION

No studies on the exposure and absorption of epichlorohydrin by humans have been reported. However, there are studies that indicate rats absorb epichlorohydrin following oral or inhalation exposure.

Weigel et al. (1978) administered a single 10 mg/kg dose of ^{14}C-epichlorohydrin by oral gavage to 21 male (mean weight 250 g) and 21 female (mean weight 208 g) Charles River CD rats. The epichlorohydrin was radiolabeled in both the carbon 1 and 3 positions and had a specific activity of 1.66 mCi/mmole. Animals were killed at 2, 4, 8, 12, 24, 48, and 72 hours after dosing, and the concentrations of radioactivity in tissues, fluids, and excreta were measured. Eight hours after treatment, less than 10 percent of the administered dose was recovered from the gastrointestinal tract. Peak tissue concentrations of radioactivity were reached 2 hours after dosing in males and after 4 hours in females. Following absorption, ^{14}C-labelled metabolites were released from the body via the urine, exhaled air, and feces (see Section 4.4).

Smith et al. (1979) administered epichlorohydrin to rats by the oral or inhalation routes and studied the pharmacokinetics of absorption, distribution, and excretion. Single oral doses of 1 mg/kg or 100 mg/kg 1,3-^{14}C-epichlorohydrin were administered to groups of four male Fischer 344 rats (weighing 190-220 g). Addi-tional groups of rats were exposed for 6 hours to air containing 1 ppm or 100 ppm 1,3-^{14}C-epichlorohydrin. The total uptake, calculated by summing all recovered radiolabel during and after the 6-hour exposure, was 15.5 μg/hour for exposure at 1 ppm and 1,394 μg/hour for exposure at 100 ppm epichlorohydrin. The doses absorbed were 0.37 and 33 mg/kg, respectively. Thus, a 100-fold difference in exposure concentration produced a 90-fold difference in the absorbed doses. At 72 hours, regardless of dose level or route, 46-54 percent of the radiolabel was excreted in the urine and 25-42 percent was exhaled as carbon dioxide. These experiments indicate that epichlorohydrin is absorbed well from the gut or the lungs, is rapidly distributed to other tissues, and much of the administered epichlorohydrin is metabolized and excreted within 72 hours.

Mice have been exposed to epichlorohydrin by the intraperitoneal and dermal routes of exposure. In a study by De Petrocellis, et al. (1982), male mice were given epichlorohydrin dissolved in corn oil, by a single intraperitoneal injection. Groups of ten mice were killed by decapitation at 1, 3, 5, 7, 10, 15, 20 and 30 minutes after injection and blood samples were collected. Levels of epichlorohydrin in the samples were determined by a gas chromatograph with a flame ionization detector; these are plotted against time in Figure 4-1. As can be seen from the graph, the in vivo half-life of epichlorohydrin is extremely short, being only just detectable after 15 minutes.

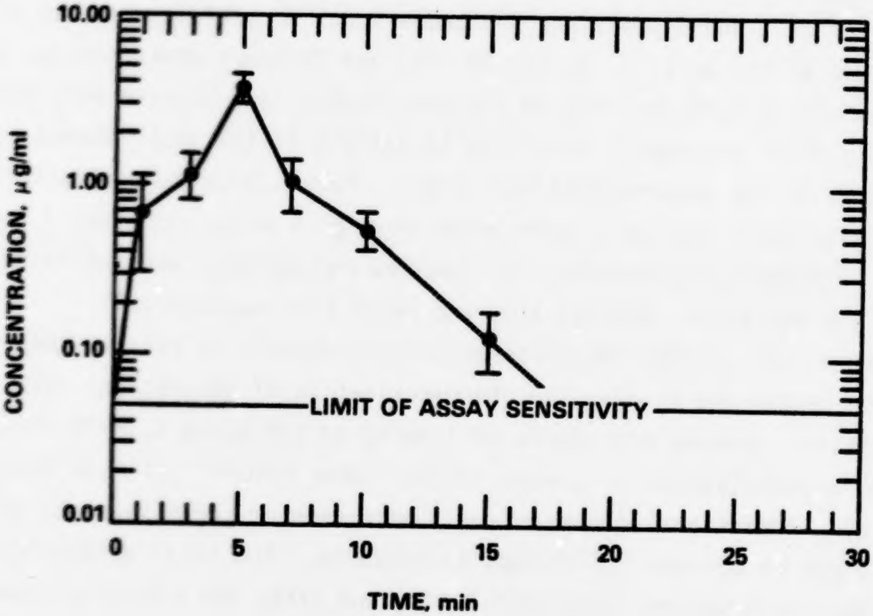


Figure 4-1. Blood concentrations of epichlorohydrin in mice after intraperitoneal injection of 200 mg/kg. Source: De Petrocellis, et al. 1982.

4.2 DISTRIBUTION

The tissue distribution of ^{14}C in rats receiving a single oral dose of ^{14}C-epichlorohydrin (10 mg/kg) was studied by Weigel et al. (1978). Details of the experiment have been outlined in Section 4.1. With the exception of kidney levels in females at 48 hours, the highest tissue concentration at all time intervals studied was in the kidneys, followed by the liver, pancreas, adrenals, and spleen. In other organs studied (lungs, heart, brain, fat, muscle, skin, ovaries, and/or testes), the levels were essentially at or below whole blood levels. Peak tissue levels were reached in male rats at 2 hours and in female rats at 4 hours. Table 4-1 shows the peak tissue levels and the 72-hour tissue levels in male and female rats. The tissue distribution patterns were similar in both sexes. The authors did not estimate rates of clearance from tissues, although data were collected at various time points. The chemical form of the radioactivity in tissues was not determined. The principal route of elimination was via the kidneys.

TABLE 4-1. DISTRIBUTION OF ^{14}C-RADIOACTIVITY IN RAT TISSUES FOLLOWING A 10 mg/kg ORAL DOSE OF ^{14}C-EPICHLOROHYDRIN

	Peak Level (ug/g)[a]		72-Hour Level (ug/g)[a]	
Tissue	Male[b]	Female[c]	Male	Female
Kidneys	22.02	22.73	4.17	8.68
Liver	11.29	7.77[d]	2.48	3.40
Pancreas	10.26	8.74	1.31	1.52
Adrenals	7.94[e]	10.55[d]	0.69	4.81
Spleen	6.87	4.81	1.30	1.56
Others[f]	2.57-4.82	2.61-5.13	0.71-1.66	0.74-1.39

[a] Epichlorohydrin equivalents.

[b] Two-hour sample.

[c] Four-hour sample.

[d] Peak value was at 8 hours.

[e] Peak value was at 4 hours.

[f] Lungs, heart, brain, fat, muscle, skin, ovaries, and/or testes.

Source: Weigel et al. (1978).

Smith et al. (1979) studied the distribution of radioactivity into 27 different tissues in Fischer 344 rats 3 hours after a single oral dose of 100 mg/kg ^{14}C-epichlorohydrin or at the end of a 6-hour exposure to 100 ppm ^{14}C-epichlorohydrin in air. After the oral dose, the highest tissue levels were found in the stomach, small intestine, kidneys, and large intestine. After inhalation, the highest levels were found in the nasal turbinates, lacrimal glands, kidneys, large intestine, and liver. The data are summarized in Table 4-2.

In this study, Smith et al. (1979) found high local concentrations in the nasal turbinates after inhalation and in the stomach after ingestion. The distribution of radioactivity in the other organs examined was similar to that found by Weigel et al. (1978).

4.3 METABOLITE IDENTIFICATION AND PATHWAYS

Smith et al. (1979) chromatographed by ion-exclusion the urinary metabolites produced after inhalation exposure or oral administration of 1,3-^{14}C-epichlorohydrin to rats. After inhalation, six peaks of radioactivity appeared in the urine, whereas after oral administration, seven peaks of radioactivity appeared in the urine (72 hours). Two major peaks separated from the urine after oral administration accounted for 23 and 10 percent of the administered radioactivity, respectively. After inhalation exposure, three major peaks in the urine accounted for about 36 percent of the radioactivity. Although there appears to be a difference in metabolism depending on route of administration, no definitive conclusions can be made, since the chemical identities of the urinary metabolites were not reported.

Fakhouri and Jones (1979) dosed male Sprague-Dawley rats orally for 5 consecutive days with 50 mg/kg epichlorohydrin and collected urine for 7 days. Ether extracts of urine were chromatographed on thin-layer plates, and the metabolites recovered were identified by gas chromatography-mass spectroscopy. The N-acetyl derivatives (mercapturic acids) of 1,3-(bis-cysteiny)propan-3-ol and S-(2,3-dihydroxypropyl)cysteine were identified as major components and beta-chlorolactic acid as a minor metabolite. No quantification of metabolites was reported. The author's proposed metabolic scheme for epichlorohydrin is shown in Figure 4-2.

Epichlorohydrin has two reactive electrophilic sites, the C-1 carbon in the epoxide ring and C-3, the chlorine-bearing carbon. These carbons can behave as alkylating agents and hence can react nonenzymatically with glutathione or protein sulfhydryl groups. However, the enzymatic reaction of epichlorohydrin

TABLE 4-2. TISSUE DISTRIBUTION OF ^{14}C-RADIOACTIVITY FOLLOWING ORAL AND INHALATION EXPOSURE OF RATS TO ^{14}C-EPICHLOROHYDRIN[a]

Timed Selected Tissues	ORAL		INHALATION 6-HOUR EXPOSURE					
	100 mg/kg[b] 3-hour Post[c]		100 ppm[b] 0-hour Post[c]		100 ppm[b] 3-hour Post[c]		100 ppm[c] 24-hour Post[c]	
	ug Eq/g	Tissue / Plasma	ug Eq/g	Tissue / Plasma	ug Eq/g	Tissue / Plasma	ug Eq/g	Tissue / Plasma
Stomach	1047.99	(29.01)[d]	6.20	(0.34)	9.59	(0.92)	5.00	(0.68)
Small instestine	154.53	(4.28)[d]	20.19	(1.10)	28.99	(2.79)	15.04	(2.05)
Kidneys	110.12	(3.05)	60.40	(3.30)	42.76	(4.12)	26.18	(3.56)
Large instestine	95.61	(2.65)[d]	75.17	(4.11)	37.24	(3.58)	11.81	(1.61)
Lacrimal gland	89.01	(2.46)	70.92	(3.88)	70.44	(6.78)	95.50	(12.99)
Liver	84.07	(2.33)	51.34	(2.81)	42.82	(4.12)	24.29	(3.30)
Lungs	64.89	(1.80)[d]	21.30	(1.16)	13.69	(1.32)	8.30	(1.13)
Brain	38.17	(1.06)	19.54	(1.07)	10.48	(1.01)	5.23	(0.71)
Plasma	36.12		18.29		10.39		7.35	
Testes	34.69	(0.96)	16.59	(0.91)	8.72	(0.84)	4.43	(0.60)
Adrenals	32.05	(0.89)	32.09	(1.75)	14.03	(1.35)	13.10	(1.78)
Heart	30.92	(0.86)[d]	17.57	(0.96)				
Nasal turbinates	28.91	(0.80)[d]	94.01	(5.14)	19.79	(1.90)	13.63	(1.85)
Muscle	23.71	(0.66)	11.67	(0.64)	5.91	(0.57)	6.12	(0.83)
Fat	10.30	(0.29)	4.36	(0.24)				

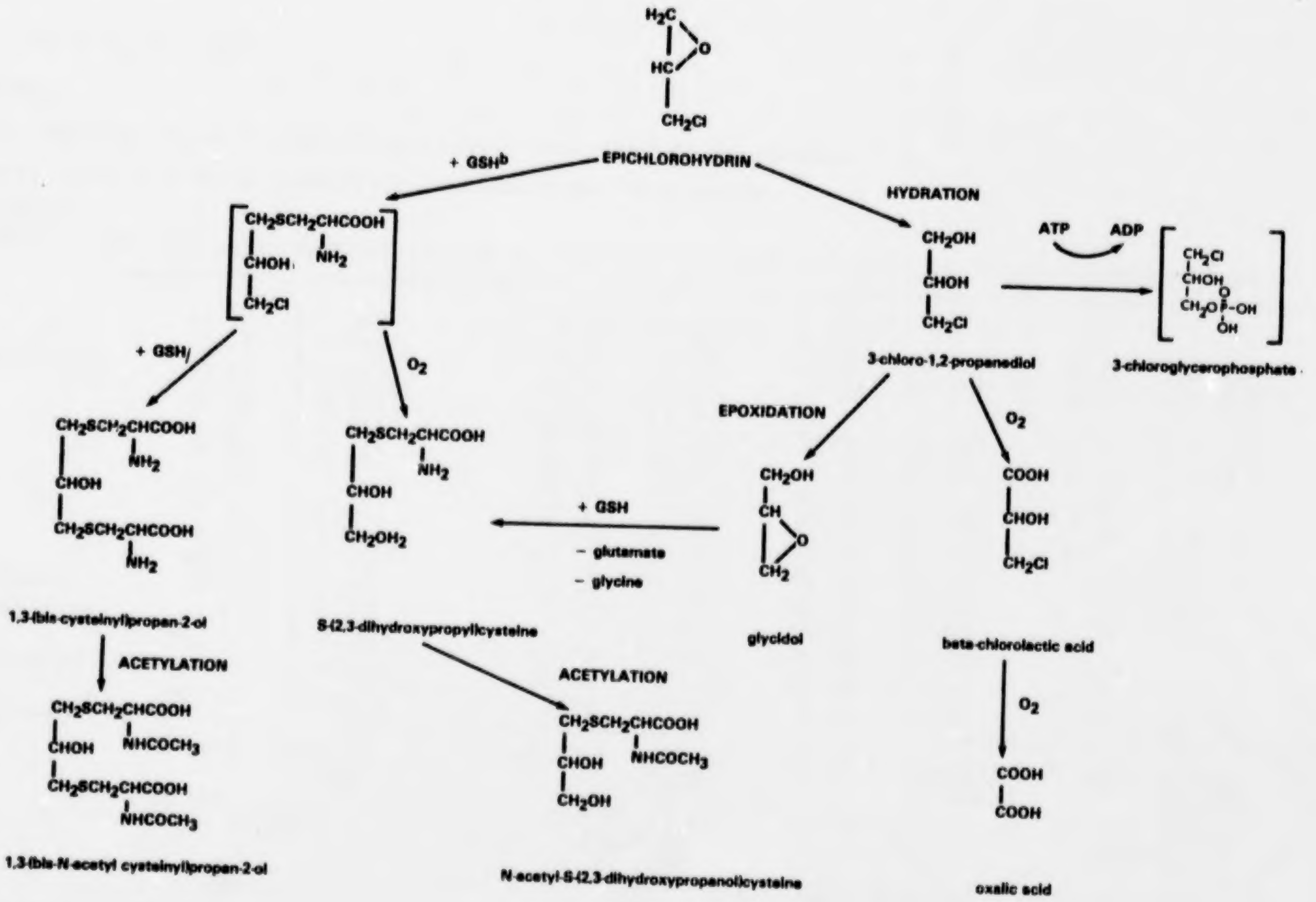
[a] Adult male Fischer 344 rats weighing 190-220 g; two rats for oral exposure and three for each of the other exposures.

[b] Dose level administered or inhalation concentration for 6 hours.

[c] Time rats were killed after dosage or after 6-hour inhalation exposure.

[d] One rat only.

Source: Smith et al. (1979).


EPICHLOROHYDRIN
+ GSH^b
HYDRATION
3-chloro-1,2-propanediol
ATP
ADP
3-chloroglycerophosphate
+ GSH
O2
EPOXIDATION
O2
1,3-(bis-cysteinyl)propan-2-ol
ACETYLATION
1,3-(bis-N-acetyl cysteinyl)propan-2-ol
S-(2,3-dihydroxypropyl)cysteine
+ GSH
– glutamate
– glycine
glycidol
beta-chlorolactic acid
O2
oxalic acid
ACETYLATION
N-acetyl-S-(2,3-dihydroxypropanol)cysteine

Compounds in brackets are hypothesized.

[b]Glutathione.

Figure 4.2 Proposed metabolic pathways for epichlorohydrin.

Source: Adapted from Fakhouri and Jones (1979) and Jones and O'Brien (1980).

with glutathione is much more rapid (Fjellstedt et al. 1973; Hayakawa et al. 1975). An enzyme, glutathione-S-epoxide transferase, isolated from rat liver, conjugates various epoxides to glutathione. Epichlorohydrin was conjugated to glutathione at 26 percent of the rate of the standard assay substrate (1,2-epoxy-3-(p-nitrophenoxy)propane) (Fjellstedt et al. 1973). The products of the enzymatic reaction were not identified; therefore, the site or extent of conjugation of epichlorohydrin glutathione was not established. (Fjellstedt et al. 1973).

Epichlorohydrin may be enzymatically converted to 3-chloro-1,2-propanediol by epoxide hydratase. Jones et al. (1969) observed that epichlorohydrin had the same antifertility effects as 3-chloro-1,2-propanediol and that both compounds resulted in the same urinary metabolite in rats, S-(2,3-dihydroxypropyl)cysteine (Fakhouri and Jones 1979; Jones and O'Brien 1980).

Fakhouri and Jones (1979) proposed that glycidol (2,3-epoxypropanol) was an intermediate in epichlorohydrin metabolism in rats. This intermediate would be formed by dehydrochlorinative cyclization of 3-chloro-1,2-propanediol. This epoxide enzymatically couples with glutathione and is converted to the mercapturic acid, N-acetyl-S-(2,3-dihydroxypropanol)cysteine, which is found in the urine of epichlorohydrin-dosed rats.

The work of Jones and O'Brien (1980), however, weakened the case for glycidol being an intermediate and suggested rather that glycidol would react with chloride ion to form 3-chloro-1,2-propanediol. This reaction would not be favored in the reverse direction (formation of glycidol). 3-Chloro-1,2-propanediol can be oxidized to chlorolactic acid. This metabolite was identified by gas-liquid chromatography of urinary methyl ester derivatives after ^{36}Cl-labeled 3-chloro-1,2-propanediol was administered to rats. Conversion of beta-chlorolactic acid to oxalic acid may occur and could result in renal toxicity due to deposition of oxalic acid crystals in the kidneys.

Jones and O'Brien (1980) also proposed that in rats 3-chloro-1,2-propanediol could be phosphorylated to 3-chloroglycerophosphate and that this compound might account for the antifertility effects of epichlorohydrin or 3-chloro-1,2-propanediol. Mashford and Jones (1978) found that 3-chloroglycerophosphate inhibited rat sperm enzyme activities (glyceraldehyde-3-phosphate dehydrogenase and triosephosphate isomerase) and hence glycolysis. Only the S(-) isomer and not the R(+) isomer of 3-chloro-1,2-propanediol produced antifertility or antiglycolytic effects. Since epichlorohydrin has not been shown to have enzyme inhibitory effects, it may be that it is metabolized in vivo to S(-) alpha-chlorohydrin phosphate, to exert its antifertility effect.

4.4 EXCRETION

The major routes of excretion of epichlorohydrin metabolites are through the urine and via the respiratory tract. Weigel et al. (1978) found that between 38 and 40 percent of the radioactivity from an oral dose of ^{14}C-epichlorohydrin was excreted in the urine of rats in 72 hours. Much of this radiolabel was excreted during the first 4 hours, 17.2 percent in male rats and 28.6 percent in female rats. Expired carbon dioxide accounted for 21 percent of the radioactivity excreted by males and 18 percent by females. The rate of conversion of label to $^{14}CO_2$ was initially rapid; by 4 hours, 8 percent of the dose and by 8 hours, 14 percent of the dose appeared as expired $^{14}CO_2$. Less than 4 percent of the radioactivity was excreted in the feces.

Smith et al. (1979) reported that 25-42 percent of the radioactivity of 1,3-^{14}C-epichlorohydrin administered orally or by inhalation was excreted as $^{14}CO_2$, while 46-56 percent of the radioactivity was excreted in the urine by 72 hours. The epichlorohydrin was administered as a single oral dose of 1 mg/kg or 100 mg/kg of 1,3-^{14}C-epichlorohydrin or as a 6-hour exposure to 1 ppm or 100 ppm in air. Approximately 2-6 percent of the radioactivity administered was recovered in the feces. The urinary metabolites were fractionated into six components by ion-exchange chromatography. None of these radiolabeled products was unchanged epichlorohydrin, and no unchanged epichlorohydrin was present in expired air.

The rate of excretion was calculated from plasma concentrations of radioactivity. After inhalation exposure to air containing 1 ppm and 100 ppm epichlorohydrin, the rate constants of excretion were 0.155 and 0.159 per hour, respectively. A semilogarithmic plot of the time course of combined excretion of radiolabel in urine and in exhaled air gives a biphasic curve, the slower phase dominating after 24 hours. The half-lives of the fast and slow phases of elimination were 1.5 and 26.4 hours, respectively. The calculated rate constants were 0.55 per hour for the fast phase and 0.26 per hour for the slow phase (Smith et al. 1979).

4.5 SUMMARY

Epichlorohydrin is well absorbed following oral, inhalation, or dermal exposure and is rapidly distributed to the various tissues and organs. The highest tissue concentrations were found in the kidneys, followed by the liver, pancreas, adrenals, and spleen. Immediately following oral administration of high doses, high tissue levels were found in the stomach, small intestine, kidneys, and large intestine; and immediately following inhalation exposure to high concentrations, high tissue levels were found in the nasal turbinates, lacrimal glands, kidneys, large intestine, and liver. The major routes of excretion of metabolites of epichlorohydrin were via the urine and respiratory tract. Approximately 40 percent of the radioactivity, regardless of the route of exposure, was excreted in the urine of rats within 72 hours. Exhaled radiolabeled carbon dioxide accounted for 18 and 21 percent of the radioactive dose in female and male rats, respectively. Fecal excretion was minor and accounted for less than 4 percent of the dose.

When epichlorohydrin was administered orally to rats, it underwent hydrolysis to produce 3-chloro-1,2-propanediol, which might be metabolized by two possible pathways (see Figure 4-2). The first was by epoxidation to glycidol, then hydrolysis and conjugation with glutathione to produce S-(2,3-dihydroxypropyl)cysteine, which was identified in rat urine.

The second pathway involved oxidation, first to chlorolactic acid and then to oxalic acid, a substance known to be toxic to the kidneys. Epichlorohydrin might also be conjugated directly with glutathione to produce S-(2,3-dihyroxypropyl)cysteine or might undergo a second conjugation with glutathione to produce 1,2-(bis-cysteinyl)propan-2-ol, which also has been identified in rat urine.

It can be concluded that epichlorohydrin in rats and mice is readily absorbed, rapidly distributed to tissues and organs, and eliminated primarily via the urine and the lungs.

5. EFFECTS ON HUMANS

Several reports of human exposure to epichlorohydrin have been found in the literature. Most were occupational exposures by inhalation or skin contact. A few experimental studies were also identified. According to one unpublished study, human exposure to epichlorohydrin has led to increased susceptibility to respiratory tract infection, altered cerebral electrical activity, cytogenic changes in hematopoietic tissues, and liver dysfunction. Epichlorohydrin is known to cause delayed skin burns, and it may cause skin sensitization reactions.

5.1 EPIDEMIOLOGIC STUDIES

Only one report of human epidemiology was identified which was unpublished in the open scientific literature. A single retropective mortality study conducted on Dow Chemical Company employees has been reported by Kilian (written communication, April 1976, as cited in NIOSH 1976a). The medical examination records of 507 employees who had been occupationally exposed to epichlorohydrin for up to 16 years were examined. The majority of these employees, however, was exposed to epichlorohydrin for 5 years or less. No control group was included in the study, and no environmental monitoring data were reported. The employees were classified as either having minimal or moderate exposures based on their job titles and work histories. An attempt was made to correlate any abnormal clinical findings with the degree of exposure. An independent consulting firm analyzed the results of the study.

The employees' records were examined for illness (work absence for 7 or more days) and for changes in electrocardiograms (ECG), chest X-rays, pulmonary function tests, and clinical chemistries including urinalysis, hemograms, and blood chemistries. Hemograms included hematocrit, leukocyte, lymphocyte, and eosinophil cell counts. Blood chemistries included creatinine and blood urea nitrogen (BUN) levels, albumin-to-globulin ratio, and lactate dehydrogenase (LDH), alkaline phosphatase (AP), serum glutamic-oxaloacetic transaminase (SGOT), and serum glutamic-pyruvic transaminase (SGPT) activities.

The illness episodes for the minimal and moderate exposure groups are shown in Table 5-1. Respiratory illness accounted for 30 percent of the illnesses reported for the moderate exposure group while employed in the epichlorohydrin exposure area, but only 12 percent of the illnesses were respiratory while workers were employed in other areas. The consulting firm concluded that the employees working in epichlorohydrin exposure areas were more likely to experience respiratory illnesses than employees working elsewhere.

Table 5-1. Illness Episodes in Epichlorohydrin Workers

	Minimal Exposure	Moderate Exposure
No. of Employees	213	49
Total Episodes of Illness	1,343	193
Episodes/Employee	6.3	4.0
Respiratory Illnesses[a]		
In Exposure Areas	254 (19%)	57 (30%)
In Nonexposure Areas	231 (17%)	24 (12%)

[a] Respiratory illnesses were tabulated while workers worked in epichlorohydrin exposure areas and in other areas as well.

Source: Adapted from NIOSH (1976a).

The electrocardiographic (ECG) and chest X-ray findings were within normal ranges as were all other clinical analyses, except for white blood cell counts at the 4th, 8th, and 12th years of employment, and eosinophil cell counts, which were slightly elevated during the 2nd and 5th years of epichlorohydrin exposure in the moderate exposure group. The LDH activities were elevated above normal levels in both exposure groups and the albumin-to-globulin ratio was significantly lower ($p<0.05$) in the moderate exposure group. The consulting firm concluded from these results that, except for the increased incidence of respiratory illness, no association could be established between epichlorohydrin exposure and pulmonary, kidney, liver, and blood effects.

This study provides useful information concerning employee exposure to epichlorohydrin and possible toxic effects; however, it is inadequate to assess properly human health hazards associated with epichlorohydrin exposure. Because of the lack of controls, the consultants compared the minimal and moderate exposure groups. No quantitative exposure data were provided, so dose-response relationships could not be developed. Also, no evaluative consideration was presented for those individuals dropped from the study because of illness, retirement, or death.

5.2 EFFECTS ON THE NERVOUS SYSTEM

Fomin (1966) determined the olfactory threshold for epichlorohydrin and examined the effects of low-level epichlorohydrin exposure on light sensitivity and cerebral electrical activity in humans. The olfactory threshold was determined in 18 subjects (sex unspecified) who were between 17 and 33 years old. The experimental details were not provided. The olfactory threshold was 0.3 mg/m^3 (0.08 ppm) in the most sensitive subjects, whereas a concentration of 0.2 mg/m^3 (0.05 ppm) was undetected by the subjects. Light sensitivity was investigated in four subjects exposed to 0.2, 0.3, 0.5, and 0.75 mg/m^3 epichlorohydrin. The experimental procedure was not described; however, for 92 dark adaptation curves obtained, no statistically significant changes in light sensitivity were observed. Fomin then examined the effects of epichlorohydrin on alpha-rhythm bursts. Two subjects were exposed to 0.2 and 0.3 mg/m^3 epichlorohydrin, and the cerebral biopotentials were recorded using an electroencephalograph (EEG). An epichlorohydrin concentration of 0.3 mg/m^3 caused significant changes in the voltage of the alpha-rhythm; in four subjects the activity increased and in one subject it decreased. No changes were observed in the subjects exposed to 0.2 mg/m^3 epichlorohydrin. The psychological and physiological significance of such alpha-rhythm changes is unclear.

5.3 EFFECTS ON BLOOD AND HEMATOPOIETIC TISSUE

5.3.1 Erythrocytes And Leukocytes

Sram et al. (1980) examined a group of 28 workers occupationally exposed to epichlorohydrin for 4 years and found decreased erythrocyte counts (3.7-4.1 x 10^{12}/l) in five workers, decreased hemoglobin concentrations (10.8-13.2 g/100 ml) in 16 workers, and decreased leukocyte counts (3.4-4.4 x 10^9/l) in five workers.

5.3.2 Peripheral Lymphocytes

A few studies were found where chromosomal abnormalities were examined in blood lymphocytes from workers occupationally exposed to epichlorohydrin. These studies are described in Section 7.2.8.3.

Kucerova et al. (1977) conducted a cytogenetic study in 35 workers occupationally exposed to epichlorohydrin for 2 years (estimated air concentration 0.5-5 mg/m^3). The percentage of cells with chromosomal aberrations was 1.37 before exposure, 1.91 after the 1st year, and 2.69 after the 2nd year of exposure. The aberrations were mostly in the form of chromatid and chromosomal breaks.

A cytogenetic evaluation of peripheral lymphocytes from 93 workers exposed to epichlorohydrin in the United States currently revealed an increase in aberration rates in comparison with a 75-person group seen for preemployment examination (Picciano 1979). Statistically significant differences were found in the distribution of individuals with chromatid breaks, chromosomal breaks, severely damaged cells, and total abnormal cells. The ratio of chromatid to chromosomal breaks for the exposed group was 4:1. These findings were consistent with the observations reported by Kucerova et al. (1977).

Recently, a cytogenetic analysis of cultured lymphocytes from 146 persons occupationally exposed to synthetic epoxy resin revealed an increase in the average frequency of cells with chromosomal aberrations (Suskov and Sazonova 1982). The synthetic epoxy resin ED-20 has epichlorohydrin as its original monomer. Individuals of both sexes were examined, and 74 healthy individuals having no occupational contacts with synthetic resins served as controls. The average age of resin-exposed workers was 39.1 years, and the period of their working with the resins ranged from 4 months to 30 years; the average age of controls was 34 years. Controls and exposed workers were matched for sex, smoking, alcohol consumption, and medications. The average concentration of epichlorohydrin in the air of work areas was determined to be 1 mg/m^3. The results of the analysis are shown in Table 5-2. The average frequency of cells with chromosomal aberrations and the number of aberrant chromosomes per cell in the exposed workers were significantly different ($p<0.001$) from those in the control workers, whereas the average frequency of breaks per aberrant chromosome did not differ significantly between the two groups.

Table 5-2. Chromosomal Aberration Frequency in Lymphocytes from Workers Exposed to Synthetic Resin ED-20

	Average Chromosomal Aberration Frequency	Aberrant Chromosomes per Cell	Breaks per Group Aberrant Chromosomes
Exposed workers	5.5%	0.054	1.23
Control workers	2.4%	0.024	1.26

Source: Suskov and Sazomova (1982).

5.3.3 Immunocompetence

Thurman et al. (1978) studied the *in vitro* effects of epichlorohydrin on human lymphocytes. Both T-cell and B-cell responses were studied. Human peripheral blood lymphocytes were separated by Ficoll-Hypaque gradient centrifugation, suspended in medium plus serum, and distributed in wells of microtiter plates. Mitogenic response was measured by incorporation of tritiated thymidine ([^{3}H]TdR) into DNA. A variety of mitogens were used: phytohemagglutinin (PGA-P, 0.05%) stimulated mainly mature T-cells; Concanavalin A (Con A, 5 ug/well) stimulated both immature and mature T-cells; pokeweed mitogen (PWM 1%) stimulated both T and B cells; and *E. coli* lipopolysaccharide (LPS, 10 ug/well) was shown to be a B-cell stimulant. Stimulated human lymphocyte cultures were exposed to 0.6, 3, 6, or 60 ug epichlorohydrin per well. At 60 ug/well, there was nonspecific cytotoxicity. At the lower levels, there was a dose-response related inhibition of the mitogenic response elicited by Con A and PWM, but not by PGA-P. No data on human lymphocytes stimulated by LPS were given. The data indicate that epichlorohydrin affects the immune function of immature lymphocytes. The *in vivo* effects on immune resistance have not been studied.

5.4 EFFECTS ON THE LIVER

Schultz (1964) reported the case of a 39-year-old worker who was accidentally exposed to epichlorohydrin gas from a tank with a defective closure. He felt paralyzed for a moment and then fled outside to the fresh air. The initial symptoms were burning of the eyes and throat that intensified after an hour. These symptoms were followed by swelling of his face, nausea, repeated vomiting, and severe headache. During the night after the exposure, he became short of breath and the next morning was admitted to the hospital. Upon examination, the mucosal lining of the upper respiratory tract was found to be irritated and the liver was enlarged. Two days following the accident, he had a significantly enlarged liver and jaundice. The serum bilirubin was 3.44 mg/100 ml, which is almost three times the upper limit of the normal range, and urobilinogen was present in the urine. After 18 days of hospitalization, the jaundice had subsided, and the patient was discharged with a slightly enlarged liver. Five months later, the patient was found to have bronchitis, elevated blood pressure, and liver dysfunction. Liver function continued to be abnormal 8 months later, when serum bilirubin was 2.6 mg/100 ml and there were abnormal amounts of urobilin and urobilinogen in the urine. The patient was examined 2 years after the exposure and found to have delayed sulfobromophthalein elimination, increased galactose excretion, and biliary pigments in the urine. Urobilin, bilirubin, and urobilinogen were positive. The liver pathology on biopsy was described as diffuse, severe, fatty degeneration. Other possible causes of liver damage and prior liver disease were explored and ruled out. It was concluded that the liver damage was caused by epichlorohydrin exposure. Chronic asthmatiform bronchitis was present and was also attributed to epichlorohydrin. In addition, the patient had hypertension that was considered to be unrelated to the exposure. No other reports of liver damage in humans exposed to epichlorohydrin were found in the literature.

5.5 EFFECTS ON THE SKIN

5.5.1 Case Studies

Ippen and Mathies (1970) described five male workers with burns resulting from exposure to epichlorohydrin or a mixture of epichlorohydrin and methanol. Two of the subjects were exposed twice.

A 25-year-old chemical worker spilled a mixture of epichlorohydrin-methanol on both hands. Two days later he noticed redness and burning of his hands. On the third evening, it had intensified so he went to an outpatient clinic. There was severe reddening and swelling of the hands to the wrists and several blisters a few millimeters in diameter. The patient was treated with corticoid ointment and Ronicol tablets (3-hydroxymethyl pyridene tartrate, a vasodilator). He returned to work 22 days later; his hands were still red 43 days after the exposure, and he had red blisters on his wrists.

A 29-year-old male accidentally spilled epichlorohydrin on his right trouser leg. Ten minutes later, he felt a burning sensation on the upper thigh of his right leg and observed mild reddening. He treated himself with an anesthetic ointment and continued to work, but 62 hours after the accident he went to an outpatient clinic because the reddening and burning continued to increase. There were two areas of deep redness the size of the palm of the hand and several smaller spots on the anterior surface of the thigh. The skin over the areas appeared dry and tanned. The worker was treated as an outpatient with antibacterial salves. He experienced no serious pain and although he still had moderate residual redness, he was able to return to work 9 days after the exposure.

A third case involved a 19-year-old male chemical worker who spilled pure epichlorohydrin on his left shoe. Six hours later, he noticed red spots on the dorsum of his foot. Burning, itching, blisters, and skin erosion developed; the blisters were opened by a physician, and the man was treated by application of a topical anesthetic and a corticoid salve. The reddening intensified, and the man was admitted to the hospital 10 days after exposure. A severe skin erosion 5 cm in diameter was observed on the dorsum of the foot. Lymph nodes in the left groin were painful and enlarged. Temperature was slightly elevated and antistreptolysin titer negative. Staphylococcus aureus was cultured from the skin erosion. He was effectively treated with penicillin injections, compresses, and corticoid salves. The patient was discharged from the hospital 1 month after exposure. Nearly 2 years later, this same subject worked for 3 days with epichlorohydrin while wearing protective rubber gloves, onto which he spilled the chemical. During the night of the third day, he noticed burning, swelling, reddening, and blister formation on several fingers of both hands. The patient was admitted to the hospital the next day. After treatment with metal foil bandages and bland salves, the lesions lessened. The patient was discharged from the hospital 10 days after admittance and returned to work 20 days later. In a followup examination 8 days after discharge, his fingers still showed persistent redness.

A fourth case involved a 32-year-old male chemical worker who accidentally poured an unspecified amount of epichlorohydrin into his right safety shoe. Even though he removed the shoe immediately and rinsed his foot with lukewarm water, a spotty redness developed over the ball and base joint of the large toe. He was admitted to the hospital within 2 hours and treated with saline solution compresses. The symptoms lessened and he was discharged after 5 days. Eight days later, he spilled epichlorohydrin into his left shoe. Despite being aware of a slight burning sensation in his foot during the night, he worked the following day. On the 3rd day after the accident, a blister developed. He was admitted to the hospital with reddening and swelling of the left foot and a blister of 2 x 1 cm filled with yellow fluid. During his hospital stay, peripheral arteriosclerosis with hyperlipidemia was diagnosed. The values for total lipids, esterified fatty acids, and triglycerides were elevated two to threefold above normal. It was concluded that no causative relationship between exposure to epichlorohydrin and the arteriosclerosis with hyperlipidemia could be determined. The symptoms regarding the affected foot subsided after local treatment with saline solution and ointments. The patient was released from the hospital after 14 days. He returned to work 4 weeks after the accident.

The fifth case involved a 21-year-old male worker who wiped up approximately 1.5 liters of an epichlorohydrin-methanol mixture (4:6) from a laboratory floor. Even though he washed his hands with soap and water, he experienced redness and itching on the palms. Four days after the incident, he began to apply a corticoid salve. He was admitted to the hospital 2 days later complaining of intense itching on both hands and red and swollen fingers. During his 11-day stay, he was treated by intravenous injections of a saponin mixture (Reparil) and by application of a heparinoid salve. The redness and swelling gradually diminished, but the surfaces of his hands were hard and rough. He did not return for followup examinations.

In the two cases in which the patients were involved in two accidental exposures, Ippen and Mathies (1970) stated that there were no signs of sensitization and referred to the skin effects as protracted chemical burns that did not develop as quickly as acid or base burns. Since the burns had a latent period of several minutes to several hours, they were more similar to burns produced by X-ray or ethylene oxide. Since epichlorohydrin can penetrate rubber or leather, specific work precautions are necessary. The severity of the burns depend on the duration and extent of exposure; therefore it would appear that there is a longer latent period for appearance of symptoms when methanol-epichlorohydrin mixtures are the causative agent (2-4 days) rather than epichlorohydrin alone.

5.5.2 Sensitization

Ippen and Mathies (1970) did not find sensitization in patients who had two exposures to epichlorohydrin. Only one human sensitization experiment (Fregert and Gruvberger 1970, as cited in NIOSH 1976a) was found in the literature. This study involved only one subject and experimental details were lacking; thus, no general conclusions could be drawn concerning skin sensitization in humans. However, some studies examining occupational eczema indicate that sensitization reactions may occur after chronic exposure to plastics and solvents containing epichlorohydrin causes skin sensitization reactions (see Section 6.1.1.6).

Jirasck and Kalensky (1960) studied patients with occupational eczema. All of the 57 patients studied were found to be hypersensitive to epoxide resins (a 20 percent solution in acetone); 23 of the 57 had weak-to-moderate reactions in skin tests with a 1 percent solution of epichlorohydrin.

Fregert and Gruvberger (1970, as cited in NIOSH 1976a) studied sensitization to epichlorohydrin and cross-sensitization to propylene oxide in one subject. Patch testing with 0.1, 0.5, and 1.0 percent epichlorohydrin in ethanol gave positive reactions after 8-11 days. Solutions of 0.1 and 0.01 percent epichlorohydrin gave positive patch tests after 2 days. Propylene oxide was positive at 0.2 percent; negative cross-sensitization results were found with chloropropane, 1-chloro-2 propanol, and ethylene oxide.

Lambert et al. (1978) presented four classes of occupational eczema where there was an allergic skin reaction to epichlorohydrin. The first case involved a subject who had worked in a chemistry laboratory for 16 years and was exposed to several chemicals including resins and epichlorohydrin. He had eczematous lesions on his hands that spread to his forearms and legs. The condition subsided when he was on vacation and was aggravated when he returned to work. Epicutaneous testing with 1 percent epichlorohydrin gave a strong-positive reaction; 0.5 percent epichlorohydrin gave a weak reaction. A second worker who molded epoxy resins developed eczema and had a moderate skin reaction to 1 percent epichlorohydrin. A third worker who had eczema on both hands had strong-positive allergic skin reactions to furan resin and epichlorohydrin. A fourth worker who had eczema on the fingers and backs of his hands manufactured fiberboard. He was not allergic to the material alone or to the solvent alone but had a positive reaction to both. The solvent was found to contain epichlorohydrin (extracted from the material). A patch test for epichlorohydrin was also positive.

5.6 SUMMARY

Epichlorohydrin has been shown to cause respiratory, skin, and eye irritation in humans. Most human exposures reported in the literature were employment related. In a single retrospective mortality study by Kilian (written communication April 1976, as cited in NIOSH 1976a), medical records for 507 employees who had been occupationally exposed to epichlorohydrin for up to 16 years were examined. Although the available information in many aspects of this study was limited, the study indicated an increase in acute respiratory illnesses in employees working in epichlorohydrin exposure areas; no relationship was noted between epichlorohydrin exposure and pulmonary, kidney, liver, and blood effects. High-level accidental exposures have produced pulmonary and liver changes in humans. In a severe epichlorohydrin inhalation poisoning, initial irritation of the eyes and throat was followed by chronic asthmatic bronchitis and extensive fatty infiltration and degenerative changes in the liver.

Headache, nausea, and head and chest congestion have been reported following worker exposure to epichlorohydrin. Local dermal contact has been shown to cause severe skin irritation. Skin burns have been reported from accidental exposures, and a few cases of skin sensitization reactions have also been reported.

Cytogenetic studies of workers exposed to epichlorohydrin have produced evidence for clastogenic effects on lymphocytes. In a recent study by Sushov and Sazonova (1982), where cultured lymphocytes from 146 workers occupationally exposed to epichlorohydrin resin were examined, the average frequency of cells with chromosomal aberrations and the number of aberrant chromosomes per cell increased significantly over controls. Epichlorohydrin should be considered as potentially hazardous to humans as a result of its mutagenic action in experimental systems and its potential to induce chromosomal effects in humans.

Limited epidemiologic studies have not demonstrated epichlorohydrin to be carcinogenic to humans; however, in long-term animal studies the compound has been shown to induce local sarcomas in mice receiving subcutaneous injections and to induce squamous cell papillomas and carcinomas of the nasal epithelium in rats exposed by inhalation. Further study of the potential carcinogencity of epichlorohydrin to mammalian species is basic to any analytical health assessment of this compound.

6. ANIMAL TOXICOLOGY

6.1 SPECIES SENSITIVITY

Most of the toxicologic information on epichlorohydrin concerns acute exposure either by inhalation or by the oral route. Only a few investigators have examined the subchronic and chronic effects of epichlorohydrin exposure in laboratory animals. No individual strain or species differences in sensitivity have been observed in the studies examined. Epichlorohydrin has been found to be extremely irritating to skin, nasal mucosa, and eyes upon acute exposure. The target organs or tissues to the subchronic effects of epichlorohydrin, in descending order of sensitivity are the nasal mucosa (when administered by inhalation route), kidneys, liver, cardiovascular system, skin, and muscle.

6.1.1. Acute Toxicity

Acute exposure to epichlorohydrin causes systemic toxicity, and, regardless of the route of exposure, results in a similar sequence of symptoms. Animals exposed to high doses of epichlorohydrin show central nervous system depression with death occurring due to paralysis of the respiratory center. A summary of the acute toxicity data is given in Table 6-1.

6.1.1.1 Inhalation--Carpenter et al. (1949) exposed groups of six male or female Sherman rats weighing 100-150 g to epichlorohydrin at a concentration of 250 ppm (950 mg/m^3 for 4 hours). The authors found that from two to four rats died in each group during exposure. The animal deaths in this study were listed as ranges rather than as specific numbers of deaths. Smyth and Carpenter (1948) and Weil et al. (1963) (apparently reporting the same data) indicated that four of six Sherman rats died after exposure to epichlorohydrin at a concentration of 250 ppm for 8 hours (time of death not specified). In these range-finding studies, the epichlorohydrin vapor was produced by injecting liquid at a metered rate into a heated Pyrex evaporator tube supplied with metered, forced air. The vapor was then cooled. The animals were exposed in a desiccator connected to the evaporator. The concentrations of epichlorohydrin were calculated on the basis of the rates of liquid delivery and airflow. In these studies, no quantitative analyses were performed on the vapor in the exposure chambers.

TABLE 6-1. ACUTE EFFECTS OF EPICHLOROHYDRIN

Route	Species	Dose	Effect	Reference
Inhalation	Rat	250 ppm for 4 h	2-4/6 dead	Carpenter et al. (1949)
Inhalation	Rat	250 ppm for 8 h	4/6 dead	Weil et al. (1963)
Inhalation	Rat	273-316 ppm for 2 h	Not lethal	Kremneva (1960)
Inhalation	Rat	360 ppm for 6 h	50% mortality	Laskin et al. (1980)
Inhalation	Rat	590-944 ppm for 2 h	62% mortality	Kremneva (1960)
Inhalation	Rat	631 ppm for 4 h	50% mortality	Grigorowa et al. (1977)
Inhalation	Rat	1,062-1,416 ppm for 2 h	80% mortality	Kremneva (1960)
Inhalation	Rat	1,416-2,124 ppm for 2 h	Lethal concentration	Kremneva (1960)
Inhalation	Mouse	237-316 ppm for 2 h	Not lethal	Kremneva (1960)
Inhalation	Mouse	590-594 ppm for 2 hr	40% mortality	Kremneva (1960)
Inhalation	Mouse	789 ppm for 2 hr	50% mortality	Grigorowa et al. (1977)
Inhalation	Mouse	1,062-1,416 ppm for 2 hr	93% mortality	Kremneva (1960)
Inhalation	Mouse	1,416-2,124 ppm for 2 hr	Lethal concentration	Kremneva (1960)
Inhalation	Mouse	2,370 ppm for 1 hr	Not lethal	Freuder and Leake (1941)
Inhalation	Mouse	7,414-16,600 ppm for 0.5 hr	Lethal concentration	Freuder and Leake (1941)
Inhalation	Mouse	18,097 ppm	50% mortality in 9.13 min	Lawrence et al. (1972)

TABLE 6-1. (continued)

Route	Species	Dose	Effect	Reference
Oral	Rat	65 mg/kg	Polyuria, proteinuria, reduced urinary chloride	Shumskaya and Karamzina (1966)
Oral	Rat	125 mg/kg	Polyuria, proteinuria, reduced urinary chloride, increased creatinine	Shumskaya and Karamzina (1966)
Oral	Rat	248 mg/kg	LD_{50}	Smyth et al. (1962)
Oral	Rat	250 mg/kg	Polyuria, proteinuria, increased urinary chlorides and creatinine	Shumskaya and Karamzina (1966)
Oral	Rat	260 mg/kg	LD_{50}	Lawrence et al. (1972)
Oral	Rat	325, 500 mg/kg	LD_{100}	Kremneva (1960)
Oral	Mouse	236 mg/kg	LD_{50}	Lawrence et al. (1972)
Oral	Mouse	250 mg/kg	Not lethal	Kremneva (1960)
Oral	Mouse	271 mg/kg	Not lethal	Freuder and Leake (1941)
Oral	Mouse	325, 350, and 590 mg/kg	LD_{100}	Kremneva (1960); Freuder and Leake (1941)
Intraperitoneal	Rat	118 mg/kg	LD_{50}	Lawrence et al. (1972)
Intraperitoneal	Mouse	165 mg/kg	LD_{50}	Lawrence et al. (1972)
Intraperitoneal	Guinea Pig	118 mg/kg	LD_{50}	Lawrence et al. (1972)
Intraperitoneal	Rabbit	165 mg/kg	LD_{50}	Lawrence et al. (1972)
Subcutaneous	Rat	65 mg/kg	Polyuria, proteinuria, reduced urinary chlorides	Shumskaya and Karamzina (1966)

TABLE 6-1. (continued)

Route	Species	Dose	Effect	Reference
Subcutaneous	Rat	100 mg/kg	Oliguria	Pallade et al. (1967)
Subcutaneous	Rat	125 mg/kg	LD_{50}, anuria, oliguria, serum protein and sodium reduced, serum potassium increased	Pallade et al. (1967)
Subcutaneous	Rat	150-180 mg/kg	66% mortality, anuria, oliguria, carbonic hydrase reduced, blood catalase reduced, lung and kidney changes	Pallade et al. (1967) Rotaru and Pallade (1966)
Subcutaneous	Rat	250 mg/kg	Polyuria, proteinuria, urinary chlorides, blood and urine creatinine increased	Shumskaya and Karamzina (1966)
Subcutaneous	Rat	500 mg/kg	Increased free aromatic amines, decreased histaminase activity	Shumskaya and Karamzina (1966)
Intravenous	Cat	9.3 mg/kg	Blood pressure decreased	Freuder and Leake (1941)
Intravenous	Cat	93 mg/kg	Minimum lethal concentration	Freuder and Leake (1941)
Intravenous	Dog	9.3 mg/kg	Blood pressure decreased	Freuder and Leake (1941)
Intravenous	Dog	93 mg/kg	Minimum lethal concentration	Freuder and Leake (1941)

TABLE 6-1. (continued)

Route	Species	Dose	Effect	Reference
Dermal (single application)	Rat	0.5 ml/kg	Not lethal	Freuder and Leake (1941)
Dermal (single application)	Rat	1,180 mg/kg for 1 h	20% dead	Freuder and Leake (1941)
Dermal (single application)	Rat	2,360 mg/kg for 1 h	90% dead	Freuder and Leake (1941)
Dermal (tail immersion, 60 min)	Mouse	---	70% dead	Pallade et al. (1967)
Dermal (tail immersion, 2 or 3 x, 20-30 min)	Mouse	---	100% dead	Kremneva and Tolgskaya (1961)
Dermal	Guinea Pig	4,420 mg/kg for 1 h	Not lethal	Freuder and Leake (1941)
Dermal	Rabbit	11.8 mg	Mild irritation	Smyth et al. (1962) Weil et al. (1963)
Dermal	Rabbit	118, 236 mg for 2 h	Lesion size, duration, intensity less than 0.5 ml	Pallade et al. (1967)
Dermal	Rabbit	590 mg for 24 h	Edema, necrosis	Pallade et al. (1967)

TABLE 6-1. (continued)

Route	Species	Dose	Effect	Reference
Dermal	Rabbit	755 mg/kg for 24 h	LD_{50}	Lawrence et al. (1972)
Dermal	Rabbit	1,180 mg for 24 h	LD_{50}	Smyth et al. (1962) Weil et al. (1963)
Dermal	Rabbit	0.2 ml of 0.3125%, 0.625%, 1.25%, 2.5%, 5.0% in cottonseed oil	No irritation to marked irritation, dose related	Lawrence et al. (1972)
Intradermal	Rabbit	0.2 ml of 0.002%, 0.008%, 0.031%, 0.125%, 0.5% in cottonseed oil	No irritation to marked irritation, dose-related	Lawrence et al. (1972)
Corneal	Rabbit	1 drop, undiluted corneal clouding, swelling	Blepharospasm, constriction	Kremneva and Tolgskaya (1961)
Corneal	Rabbit	1 drop, undiluted corneal injury	Grade 4, moderately severe	Smyth et al. (1962)
Corneal	Rabbit	0.1 ml, 40% in cottonseed oil	Iritis, palpebral irritation, edema	Lawrence et al. (1972)
Corneal	Rabbit	0.1 ml, 20% in cottonseed oil	Conjunctival and palpebral irritation, edema	Lawrence et al. (1972)
Corneal	Rabbit	0.1 ml, 10% in cottonseed oil	Dubious irritation	Lawrence et al. (1972)
Corneal	Rabbit	0.1 ml, 5% in cottonseed oil	No irritation	Lawrence et al. (1972)

The median lethal concentration for acute epichlorohydrin inhalation exposure was determined by Laskin et al. (1980). Groups of 20 male Sprague-Dawley rats were exposed to epichlorohydrin concentrations ranging from 283 to 445 ppm (1,075 to 1,691 mg/m^3) for 6 hours. The animals were then observed for mortality over 14 days. No deaths were observed at 283 ppm. Only one death (5 percent) was observed at both 303 and 339 ppm (1,151 and 1,288 mg/m^3). At higher concentrations, mortality increased sharply; at 369 ppm (1402 mg/m^3), 15 of the 20 exposed animals died (75 percent) within 14 days after exposure. At 421 and 445 ppm (1,600 and 1,691 mg/m^3), 16 and 17 animals died, respectively. From these data, the 14-day LC_{50} for epichlorohydrin was estimated to be approximately 360 ppm. Pathologic examination of four animals from each exposure group revealed acute respiratory tract irritation, hemorrhage, and severe pulmonary edema. The lung-to-body weight ratios were determined, and marked elevations were observed at the higher exposure levels. For example, at 369 ppm epichlorohydrin, an 80 percent increase in lung-to-body weight ratio was detected when compared with controls. At the lowest two exposure levels (283 and 303 ppm), there were no increases over controls in lung-to-body weight ratio.

Freuder and Leake (1941) exposed white mice (unspecified strain, sex, and age) to epichlorohydrin by inhalation at concentrations of 2,370 ppm for 60 minutes and at 8,300 (31.54 g/m^3) and 16,600 (63.1 g/m^3) ppm for 30 minutes. In the group of 30 animals exposed at 2,370 ppm, no mortality was observed within 24 hours after exposure. However, in the group of 20 animals exposed at 8,300 ppm and in the group of 30 animals exposed at 16,600 ppm, all the animals died. All animals exposed to epichlorohydrin showed irritation of the nose and eyes. "Delirium" was observed 3 minutes after exposure began at 16,600 ppm and within 14 minutes after exposure to 8,300 ppm. At the two highest exposure levels, the animals first became quiescent and then developed cyanosis and muscular relaxation of the extremities. This was followed by tail stiffening and fine tremor of the body. The respiration became increasingly depressed. Some animals experienced clonic convulsions. The animals exposed to epichlorohydrin at 2,370 ppm showed no symptoms of toxicity other than nose and eye irritation.

Kremneva (1960) and Kremneva and Tolgskaya (1961) studied the acute inhalation toxicity of epichlorohydrin in both white rats and mice (strain, age, weight, and sex unspecified). The rats and mice were exposed for a single 2-hour period in a 100-liter chamber. The epichlorohydrin was placed in the chamber as a liquid and allowed to evaporate. Air samples were withdrawn from the chamber between 15 and 30 minutes and then again at 90 minutes for analysis. After reaching a maximum value, the concentration of epichlorohydrin fell to nearly half of the initial value during the 2-hour exposure period. The animals were observed for 14 days following exposure. The results of these studies are shown in Table 6-2. All deaths, except for that of one mouse, occurred within the first 3 days after exposure.

Table 6-2. Summary of Mortality Findings in Rats and Mice after Acute Inhalation Exposure to Epichlorohydrin

Range of Concentration		No. of Test Animals		Mortality (%)	
mg/m^3	ppm	Rats	Mice	Rats	Mice
899-1,199	237-316	15	15	0	0
2,242-3,587	590-944	18	20	55	40
4,036-5,381	1,062-1,416	10	15	80	93
5,381-6,278	1,416-1,652	10	10	100	100
6,726-8,071	1,770-2,124	10	10	100	100

Source: Kremneva (1960); Kremneva and Tolgskaya (1961).

The authors stated that rats and mice appeared to have essentially identical sensitivities to inhaled epichlorohydrin vapor. The lethal concentration for both rats and mice was 1,416 ppm (5,381 mg/m^3) and the LC_{50} ranged from 590 to 944 ppm (2,240 to 3,587 mg/m^3). The maximum concentration that produced no observable signs of toxicity was 316 ppm (1,199 mg/m^3). Epichlorohydrin caused irritation of the mucous membranes of the upper respiratory tract, initial stimulation followed by depressed activity, increasingly depressed respiration, and dyspnea resulting in asphyxia. Cutaneous hyperemia and areas of subcutaneous hemorrhage were observed. No loss of righting reflex was observed during the 2-hour exposure periods. Death from progressive respiratory dysfunction occurred several hours following exposure. Microscopic examination of tissues and organs from dead animals revealed inflammatory desquamative bronchitis, necrosis of the bronchial mucosa, and pulmonary edema. The kidneys showed degeneration and necrosis of the convoluted tubules and glomerular edema. Hemorrhagic changes were observed in the mucosa of the stomach and the small intestine. Sections of the myocardium showed fibers that were disorganized and fragmented.

Lawrence et al. (1972) determined an LT_{50} (lethal time 50 percent) for male ICR mice exposed to air saturated with epichlorohydrin vapor. Groups of mice were placed in an 8.75-liter glass chamber. The air in the chamber was saturated with epichlorohydrin by bubbling air through liquid epichlorohydrin and then passing the air into the chamber. The concentration of epichlorohydrin in the chamber was calculated by dividing the weight loss of the liquid by the quantity of air passed through the liquid. Groups of mice were exposed for specific time intervals, and then observed for 7 days. The LT_{50} was determined to be 9.13 minutes, with 95 percent confidence limits of 8.49-9.81. At 9.13 minutes, the exposure chamber should have reached an 88 percent equilibrium with the saturated vapor entering the chamber. At a room temperature of 23°C and a barometric pressure of 30.18 inches of mercury, the epichlorohydrin concentration was calculated to be 71.89 mg/l (18,907 ppm), with a maximum deviation over three separate exposures of 1.26 mg/l.

6.1.1.2 Oral--Freuder and Leake (1941) examined the acute oral toxicity of epichlorohydrin in mice. Epichlorohydrin was suspended in a 25 percent aqueous gum arabic solution and mixed. Each animal received the same dose volume based on body weight (0.1 ml/10 g). Groups of 15 white mice (unspecified strain, sex, and age) were administered either 0.50 or 0.23 ml/kg (588 or 270 mg/kg) epichlorohydrin by stomach tube. Immediately after administration of 588 mg/kg epichlorohydrin, the mice showed intoxication (erratic movements) for a few minutes, then the erratic movements ceased and respiration slowed. A dose level of 588 mg/kg was lethal to all 15 test animals. At a dose level of 270 mg/kg, all 15 test animals survived the 24-hour observation period.

Kremneva (1960) and Kremneva and Tolgskaya (1961) examined the acute oral toxicity of epichlorohydrin administered by gavage in 30 mice and 15 rats (strain, age, weight, and sex unspecified). Epichlorohydrin was administered in aqueous solution at 250, 325, and 500 mg/kg. A dose of 250 mg/kg did not produce any observable signs of toxicity during 2 weeks of observation. The two highest doses produced mortality in both rats and mice usually within the first 48 hours after treatment. The signs of toxicity observed were lethargy, slowed respiration, subcutaneous hemorrhage, dyspnea, rales, ataxia, and tremors. Gross examination of the dead animals revealed hyperemia and hemorrhage in the lungs and other organs, and a yellow discoloration of the liver. Microscopic examination showed hemorrhages and edema in the pulmonary tissues, degenerative changes with areas of necrosis in the convoluted tubules of the kidneys, and fatty degeneration of the liver. Foci of necrosis were also observed in the mucosa of the stomach and intestine.

Lawrence et al. (1972) determined the acute oral LD_{50} of epichlorohydrin in male ICR mice and in male Sprague-Dawley rats to be 0.20 and 0.22 ml/kg (235 and 260 mg/kg), respectively. The 95 percent confidence interval was 0.16-0.25 ml/kg in mice and 0.12-0.39 ml/kg in rats. The epichlorohydrin in this study was administered by gavage in cottonseed oil.

Smyth et al. (1962) and Weil et al. (1963) determined the oral LD_{50} of undiluted epichlorohydrin in male Carworth-Wistar rats, which were 4-5 weeks old and weighed 90-120 g. Mortality observations were made for 14 days after compound administration. The LD_{50} was determined to be 0.21 ml/kg (260 mg/kg). No statistical information was provided.

6.1.1.3 Subcutaneous Injection--Several investigators have examined the acute toxicity of epichlorohydrin by subcutaneous administration. Kremneva and Tolgskaya (1961) administered epichlorohydrin subcutaneously to 50 mice (strain, age, weight, and sex unspecified) at 125, 250, 375, and 500 mg/kg. The 125 mg/kg dose was tolerated and the animals showed no observable behavioral changes. A dose of 250 mg/kg was lethal to 7 of 10 animals. Survival time was not described. The 375 and 500 mg/kg dose were lethal to all the treated animals.

Shumskaya and Karamzina (1966) reported the toxic effects of epichlorohydrin in the kidneys from a single subcutaneous injection. It was the intent of the authors in this study to investigate methods for detecting kidney dysfunction and not to examine the role of epichlorohydrin in renal toxicity. For this reason, there was little information provided concerning the experimental details such as the number of animals treated, animal observations, sample times, and the length of time before the onset of toxic effects. The authors also did not report the methods used for many of the clinical determinations. Rats (strain, sex, and age unspecified) were administered epichlorohydrin subcutaneously in single doses of 65, 125, or 250 mg/kg. Several parameters were examined in this study; however, not all parameters were reported for each dose level, so dose-response relationships could not be identified. Generally, the authors found that epichlorohydrin administered subcutaneously produced polyuria, decreased urinary specific gravity, proteinuria, decreased urinary chlorides, increased kidney-to-body weight ratios, and increased nitrogenous substances in the blood.

Rotaru and Pallade (1966) and Pallade et al. (1967) described gross and microscopic findings in rats following subcutaneous injection of epichlorohydrin. A total of 37 albino rats weighing 180-200 g (strain, sex, and age unspecified) received a single subcutaneous injection of either 150 mg (23 rats) or 180 mg (14 rats) of epichlorohydrin. No control animals were mentioned for this study. Animals were killed at 24 hours, 48 hours, 5 days, and 10 days after treatment. The number necropsied was not specified. The following tissues were examined: heart, lungs, kidneys, liver, adrenals, spleen, stomach, intestine, and brain; most affected were the kidneys. The changes observed in the kidneys at both dose levels were qualitatively similar; however, more severe changes were observed at the higher dose level (180 mg). At 24 hours after treatment, the rats examined showed kidney toxicity consisting of ischemia of the cortex, and congestion of the medulla with marked interstitial edema. Degenerative changes were observed throughout the tubules with necrotic lesions observed also in the proximal convoluted portions. Signs of regeneration were observed in the kidneys 5 days after treatment. Ten days after treatment, only a few signs of ischemic necrosis were observed, and most of the tubular integrity was restored. The changes in the other organs were not as severe as those observed in the kidneys. The lungs showed areas of congestion of the alveolar septa, desquamative bronchial inflammation, and some edema of the bronchiovascular connective tissue. The heart tissue was normal except for some limited myocardial congestion. The spleen showed stasis and some limited hemorrhage, except in one animal, where hemorrhaging was extensive. The stomach and intestines showed slight mucosal congestion and edema. The liver and adrenals appeared normal except for some limited congestion in a few animals (number unspecified).

6.1.1.4 Intraperitoneal Injection--Lawrence et al. (1972) reported LD_{50} values for several animal species for the intraperitoneal injection of epichlorohydrin. Male ICR mice, male Sprague-Dawley rats, male Hartley albino guinea pigs, and male New Zealand albino rabbits (number, age, and weight unspecified) were treated with epichlorohydrin dissolved in cottonseed oil. The LD_{50} values and confidence limits are shown in Table 6-3.

6.1.1.5 Intraveneous Injection--Freuder and Leake (1941) studied the effects of epichlorohydrin injected intravenously in cats and dogs. Three cats and two dogs (sex, age, and weight unspecified) were anesthetized with sodium pentobarbital. The blood pressure was measured from the carotid

Table 6-3. Acute Intraperitoneal Toxicity of Epichlorohydrin

Species	LD_{50} (mg/kg)	95% Confidence Limit (mg/kg)
Mouse	170	153-188
Rat	113	94.6-134
Guinea pig	118	29.5-472
Rabbit	160	83.6-306

Source: Lawrence et al. (1972).

artery. The respiration was recorded directly from the trachea by measuring pressure changes. Epichlorohydrin was suspended either in water or acacia solution before injection. The doses administered to each animal were not reported nor was the suspension vehicle nor the concentration of epichlorohydrin in the vehicle. The authors reported that cats and dogs showed similar blood pressure responses to epichlorohydrin administered intravenously. Doses of epichlorohydrin below 9.3 mg/kg were essentially inactive in affecting blood pressure or respiration; at 9.3 mg/kg, there were only transitory decreases in blood pressure. The minimum lethal concentration was approximately 93 mg/kg in both dogs and cats. Immediately after injection of a 93 mg/kg dose, there was a rapid decrease in blood pressure followed by a moderate increase. Respiration increased and deepened in the cats, whereas in the dogs there was a brief period of apnea and then an increase in respiration rate. Death occurred in both the dogs and cats within 2 hours.

6.1.1.6 Percutaneous Application--Epichlorohydrin has been shown to be irritating to the skin and is readily absorbed to cause systemic toxicity. Several investigators have examined the toxicity, irritation, and sensitization potential of epichlorohydrin in laboratory animals. Freuder and Leake (1941) studied the acute percutaneous toxicity of epichlorohydrin in white rats (strain, sex, age, and weight not specified). The abdomens of the rats were shaved, and a 1-cm square piece of gauze wetted with a measured amount of epichlorohydrin was applied to the shaved area. The gauze was removed after 1 hour. Groups of 10 rats each were exposed to 0.5 ml/kg (6.5 mmol/kg) and 1.0 ml/kg (13.0 mmol/kg) epichlorohydrin. Twenty rats were exposed to 2.0 ml/kg (26.0 mmol/kg). The observation time was unspecified; it is assumed to have been several days. At the lowest exposure level (0.5 ml/kg), all 10 rats survived. At the intermediate dose level (1.0 ml/kg), 8 of 10 rats survived; and at the highest dose level (2.0 ml/kg), only 2 of the 20 exposed rats survived. The authors noted discoloration of the skin after exposure, with occasional superficial desquamation within a few hours.

Smyth et al. (1962) and Weil et al. (1963) determined the acute dermal LD_{50} in rabbits. Groups of four male albino New Zealand rabbits weighing 2.5-3.5 kg were immobilized for a 24-hour contact period. The fur was clipped from the skin, undiluted epichlorohydrin was applied, and then the skin was covered with an impervious plastic film. After a 24-hour contact period, the film was removed and the rabbits were observed for 14 days. The approximate percutaneous dermal LD_{50} for rabbits was 1.3 ml/kg. From a toxicologic viewpoint, great care should be exercised in the interpretation of results from such experiments because restrained, conscious animals usually have high levels of catecholamines in their circulation. It is well known that catecholamines affect the cutaneous vascular tone and, hence, the rate of absorption of substances applied to the skin.

Kremneva and Tolgskaya (1961) studied the skin absorption of epichlorohydrin in mice. The tails of 20 mice (strain, sex, and age unspecified) were immersed to three-quarters of their length in epichlorohydrin for either 1 hour for a single exposure or for 20-30 minutes, 2-3 times, for a repeat exposure. The single 1-hour exposure produced signs of toxicity and death in 6 of 10 of the experimental mice. All 10 mice that received multiple exposures to epichlorohydrin died. The signs of toxicity were similar to those already described for inhalation or oral exposure. The animals showed decreased activity, increasingly depressed respiration, and loss of righting reflex. Examination of the dead animals showed congestion and edema of the internal organs, hemorrhages in the brain, and necrosis of the renal tubules.

Pallade et al. (1967) examined the percutaneous absorption of epichlorohydrin in mice. The tails of 10 mice (strain, sex, age, and weight unspecified) were immersed in epichlorohydrin for 15-20 minutes. Seven mice died within 24 hours following exposure. The effects described were similar to those observed by Kremneva and Tolgskaya (1961).

Smyth and Carpenter (1948) reported skin irritation following the application of 0.01 ml of epichlorohydrin to the clipped abdominal skin of five albino rabbits (strain, sex, age, and weight unspecified). The authors described the irritation as a slight increase in local capillary permeability.

In a study by Kremneva and Tolgskaya (1961), a small glass cap was affixed to a rabbit's back (experimental details not provided). The glass cap contained 0.5-1.0 mg epichlorohydrin. After 1 hour of exposure, the glass cap was removed and the site was washed with soap and water. The skin appeared hyperemic; then ulcerlike lesions developed followed by scabbing. Complete recovery took 1-1.5 months.

Lawrence et al. (1972) examined the dermal irritation that occurred when epichlorohydrin was applied to the shaved backs of male albino New Zealand rabbits. A "Webril" patch (1.27 cm square) was wetted with 0.2 ml of undiluted epichlorohydrin and placed on the shaved backs of rabbits and covered with an occlusive bandage for 24 hours. An 8 percent aqueous solution of sodium lauryl sulfate was used as a positive control and cottonseed oil was used as the negative control. After the patch was removed, the irritancy was evaluated on a 0 to 3+ scale. Epichlorohydrin showed considerable irritant activity. Undiluted epichlorohydrin produced irritation equal to, or greater than, the positive control (3+). The same procedure was then used with epichlorohydrin diluted with cottonseed oil; 0.2 ml volumes of the various dilutions were tested. Table 6-4 shows the results of the tests.

Lawrence et al. (1972), in determining the acute percutaneous LD_{50} for epichlorohydrin in rabbits, used the same procedure as that for the irritancy testing. Measured amounts of epichlorohydrin were placed on the "Webril" patch, and it was covered with an occlusive bandage for 24 hours and then removed. The mortality observations were recorded for 6 days. The LD_{50} value in male New Zealand albino rabbits was 0.64 ml/kg (755 mg/kg), with 95 percent confidence limits of 0.33-1.22 ml/kg (384-1,445 mg/kg).

Table 6-4. Dermal Irritation Scores for Solutions of Epichlorohydrin in Cottonseed Oil

% Epichlorohydrin (v/v)	Response
0.3125	0
0.625	±
1.25	1+
2.5	2+
5.0	3+

Source: Lawrence et al. (1972).

Lawrence et al. (1972, 1974) examined the sensitization potential of epichlorohydrin using the guinea pig maximization test. Five male Hartley albino guinea pigs weighing 300-500 g received intradermal injections of 0.01 percent epichlorohydrin in cottonseed oil and complete Freund's adjuvant. Seven days after the first injection, epichlorohydrin was applied topically over the injection site, and the site was then covered with an occlusive bandage for 48 hours. Two weeks later, the hair was shaved from a different site (hind flank) and the epichlorohydrin was applied topically and covered with an occlusive bandage for 24 hours. The bandage was then removed, and the site was cleansed with alcohol. Twenty-four hours later, the site was evaluated for sensitization reactions. No evidence of sensitization was observed in any of the five treated guinea pigs. The positive control, 25 percent 2,4-dinitrochlorobenzene, produced a response of 3^+ grade (intense redness and swelling).

Weil et al. (1963) also examined the sensitization potential of epichlorohydrin in guinea pigs. Eighteen guinea pigs (strain, sex, age, and weight unspecified) were injected intradermally with 0.1 ml of diluted epichlorohydrin (concentration unspecified) three times a week on alternate days for a total of eight injections. After a 3-week period with no exposure, a challenge dose was injected and the animals were examined 24 and 48 hours thereafter for sensitization reactions. The concentration of epichlorohydrin in the challenge dose was unspecified. Sensitization reactions were not observed in any of the treated guinea pigs.

In contrast to the above test results, when Thorgeirsson and Fregert (1977) examined the sensitization potential of epichlorohydrin using the guinea pig maximization test, positive results were observed in more than half of the animals. Fifteen female Hartley guinea pigs weighing 300-400 g were injected intradermally with 0.1 ml of equal portions of 5 percent epichlorohydrin (w/v) in ethanol and complete Freund's adjuvant. The same procedure was followed as described in the study by Lawrence et al. (1974). After 1 week, the occluded patch was wetted with 2 percent epichlorohydrin in ethanol and applied to the skin over the injection site for 48 hours. After 2 weeks, an occluded patch wetted with 1 percent epichlorohydrin solution was applied to the shaved skin at another site on the body. This sensitization test gave positive results in 9 of the 15 animals tested. The authors classified epichlorohydrin as a grade 3 or moderate sensitizer.

It is not clear from these studies whether epichlorohydrin causes skin sensitization reactions. Further animal studies are necessary before the sensitization potential of epichlorohydrin can be reliably determined. However, there are reports (Jirasck and Kalinsky 1960; Lambert et al. 1978) of skin sensitization reactions in humans occupationally exposed to epichlorohydrin (see Section 5.4.2).

6.1.2 Subchronic and Chronic Toxicity

There are few subchronic and chronic epichlorohydrin toxicity studies in the published literature. A recently completed 90-day study (Quast et al. 1979a) has been designed and conducted in a much more thorough manner than previous subchronic studies. A summary of the subchronic toxicity data appears in Table 6-5. The only chronic study found in the literature was published by Laskin et al. (1980). This study was relatively complete and well designed; however, all groups of animals, including controls, had a high incidence of respiratory tract infections and pneumonia and, also, poor survival rates.

6.1.2.1 Inhalation--Gage (1959) exposed five groups of eight albino Wistar rats each (four males and four females) to epichlorohydrin vapor at concentrations of 9, 17, 27, 56, and 120 ppm (34, 65, 103, 213 and 456 mg/m^3) daily for 6-hour periods, 5 days/week, for a total of 11 to 19 exposures. The test animals weighed between 160 and 200 g. No control animals were reported in this exposure study. The epichlorohydrin vapor was prepared by atomizing solutions of epichlorohydrin and propanol in a metered stream of air. The concentration of epichlorohydrin was calculated based on the amount of solution delivered by the feed syringe, the concentration of the epichlorohydrin in the propanol solution, and the rate of airflow to the atomizer. Daily checks were made of the chamber's atmospheric concentrations of epichlorohydrin using a colorimetric method.

Rats exposed to epichlorohydrin at 120 ppm (456 mg/m^3) showed labored breathing after the first 3 hours, which continued throughout the remaining exposures. Between exposures, the animals were lethargic and their condition progressively deteriorated during the study. Considerable loss of weight, nasal discharge, and marked leukocytosis were observed. One rat died after 11 exposures, at which time the study was terminated. At termination, the author reported that "the urinary protein was more than double the normal value", indicating possible kidney damage. At necropsy, the kidney cortex was pale in color. Microscopic examination revealed areas of leukocytic infiltration and atrophy of the peripheral cortical tubules in four of the eight animals examined. The lungs showed congestion, edema, consolidation, and inflamed areas with signs of abscess formation. Microscopic examination of the liver revealed generalized congestion with one animal's liver showing areas of necrosis.

Rats exposed to epichlorohydrin at 56 ppm (213 mg/m^3) were lethargic after the 10th exposure and later in the study exhibited respiratory distress, loss of weight, and nasal discharge. Limited recovery was evident following the weekends. Urinary protein, hemoglobin levels in blood, and differential cell counts were normal. Eighteen exposures were made at 56 ppm (213 mg/m^3) before the study was terminated. No abnormalities were observed at necropsy, and no abnormal microscopic findings were reported except for an abscess formation in one lung, which the author did not attribute to epichlorohydrin exposure.

Eighteen exposures to 27 ppm (103 mg/m^3) caused mild nasal irritation and 19 exposures to 17 ppm (65 mg/m^3) epichlorohydrin caused no adverse effects. Two rats exposed 18 times to 9 ppm (34 mg/m^3) developed pulmonary infections; however, the remaining animals in this group were healthy and had normal weight gains. In this study, no results were presented for vehicle controls (propanol); therefore, it is difficult to attribute the changes observed solely to epichlorohydrin exposure.

Gage (1959) similarly exposed two New Zealand white male rabbits weighing 1.8-2 kg to 35 ppm (133 mg/m^3) epichlorohydrin by daily inhalation for 20 days. These animals showed signs of nasal irritation, normal weight, and no abnormal gross or microscopic findings. Two rabbits exposed to 16 ppm (61 mg/m^3) epichlorohydrin showed nasal irritation after two exposures. The concentration was then decreased to 9 ppm (34 mg/m^3), and exposure continued for 20 days. No effects were observed and gross and microscopic examination of the tissues from these animals revealed no tissue changes that could be attributed to the epichlorohydrin exposure.

Kremneva and Tolgskaya (1961) exposed two groups of eight rats (strain, sex, and age unspecified) to epichlorohydrin by inhalation. The first group of animals was exposed 3 hours/day for 5 months at a concentration of

TABLE 6-5. SUBCHRONIC EFFECTS OF EPICHLOROHYDRIN

Species	Route	Dose	Effect	Reference
Rat	Inhalation	9 ppm 6 h/d X 5 d/wk 18 exposures	No effects except two pulmonary infections	Gage (1959)
Rat	Inhalation	17 ppm 6 h/d X 5 d/wk 19 exposures	Normal weight gain, inferior condition, no abnormal pathology	
Rat	Inhalation	27 ppm 6 h/d X 5 d/wk 18 exposures	Nasal irritation, constant body weight, no weight gain, no abnormal pathology except for one consolidated lung	
Rat	Inhalation	56 ppm 6 h/d X 5 d/wk 18 exposures	Weight loss, nasal irritation and discharge, respiratory distress. No effect on hemoglobin and differential cell counts or urinary protein, no abnormal pathology	
Rat	Inhalation	120 ppm 6 h/d X 5 d/wk 11 exposures	Weight loss, nasal irritation and discharge, lethargy, deteriorated condition, leukocytosis, increased urinary protein. Lungs were congested, consolidated, edematus, with inflammation and abscess formation; kidneys showed atropic tubules, leukocytic infiltration. Liver congested.	
Rat	Inhalation	20-60 mg/m^3 3 h/d X 5 d/wk X 6.5 mo	Weight gain 5-10% less than control, slightly elevated blood pressure, pathology same as 170-250 mg/m^3 except not as severe.	Kremneva and Tolgskaya (1961)

TABLE 6-5. (continued)

Species	Route	Dose	Effect	Reference
Rat	Inhalation	170-250 mg/m^3 3 h/d X 5 d/wk X 5 mo	General deterioration in condition, subcutaneous hemorrhage, respiratory distress and dyspnea, two deaths after first month, remaining animals died at the start of fifth month of exposure. Elevated blood pressure after 1-2 mo exposure. Bronchitis, necrosis of bronchial mucosa, thickened and alveolar septa, pulmonary edema. Deterioration of kidneys, necrosis of convoluted tubules. Hepatic cells showed fatty degeneration and vacuolization. Myocardial tissue stained irregularly, fragmentation of myocardial fibers.	Kremneva and Tolgskaya (1961)
Rat	Inhalation	0.2 mg/m^3 continuous exposure, 98 days	No effects observed	Fomin (1966)
Rat	Inhalation	2 mg/m^3 continuous exposure, 98 days	Decreased blood nucleic acids, no effects on erythrocytes, leukocytes, and hemoglobin levels. Increase in fluorescent dye-fixing leukocytes. No abnormal pathology.	
Rat	Inhalation	20 mg/m^3 continuous exposure, 98 days	Decreased blood nucleic acids No effects on erythrocytes, leukocytes, and hemoglobin levels. Increase in fluorescent dye-fixing leukocytes. Emphysema, desquamative interstitial pneumonia, edema and deterioration of vascular connective tissue of lung. Intermuscular, micro-focal hemorrhages and venous plethora in heart. Necrosis of the convoluted tubules of kidney. Damaged neurons in medulla oblongata, cerebellum and hippocampus.	Fomin (1966)

TABLE 6-5. (continued)

Species	Route	Dose	Effect	Reference
Rat	Inhalation	5 ppm 6 h/d X 5 d/wk 61-62 exposures	No effects	Quast et al. 1979a
Rat	Inhalation	25 ppm 6 h/d X 5 d/wk 61-62 exposures	Decreased activity, local eye irritation. Inflammatory, degenerative changes in respiratory and olfactory epithelium. One kidney tumor unrelated to exposure.	
Rat	Inhalation	50 ppm 6 h/d X 5 d/wk 61-62 exposures	Decreased activity, local eye irritation. Decreased weight gain. Inflammatory and degenerative changes in respiratory and olfactory epithelium of the nasal turbinates. Increased kidney weights. Tubular necrosis and edema of renal cortex. Decreased hepatocellular glycogen content. Increased vacuolization of cytoplasm in zona fasciculata of adrenal cortex.	
Rat	Inhalation	100 ppm 6 h/d X 5 d/wk 9 exposure in 12 days	Nasal discharge, respiratory irritation decreased food intake, decreased body weight. Degeneration, inflammation, hyperplasia, squamous metaplasia of respiratory and olfactory epithelium. Other changes same as 50 ppm exposure, except more severe.	Quast et al. 1979b

TABLE 6-5. (continued)

Species	Route	Dose	Effect	Reference
Mouse	Inhalation	2,370 ppm 1 h/d until all animals dead	All animals died after 16 exposures; half died after 7 exposures.	Freuder and Leake (1941)
Mouse	Inhalation	5 ppm 6 h/d X 5 d/wk 61-62 exposures	No effects.	Quast et al. 1979a
Mouse	Inhalation	25 ppm 6 h/d 5 d/wk 61-62 exposures	Slightly decreased weight gain. Inflammation and degenerative changes in nasal turbinates. No abnormal changes in other organs.	
Mouse	Inhalation	50 ppm 6 h/d X 5 d/wk 61-62 exposures	Decreased weight gain. Decreased food intake. Inflammation and degeneration changes in nasal turbanates. Focal subacute pneumonitis. No abnormal changes in other organs.	
Mouse	Inhalation	100 ppm 6 h/d X 5 d/wk 9 exposure in 12 days	Nasal irritation, decreased food intake, decreased body weight. Inflammation and degenerative changes in nasal turbinates. Decreased hepatocellular glycogen content. Decreased hepatocyte size. Focal subacute pneumonitis. Slight atrophy of thymus.	Quast et al. 1979b
Rabbit	Inhalation	9, 16, 30 ppm 6 h/d for 20 days	Nasal irritation, normal body weight increases, no abnormal pathology.	Gage (1959)
Rat	Oral	94 mg/kg	Administered daily until all animals died. First death after 2 doses. 100% mortality after 21 doses.	Freuder and Leake (1941)
Rat	Oral	190 mg/kg	First death after first dose. 100% mortality after 8 doses.	
Rat	Oral	271 mg/kg	First death after first dose. 100% mortality after 4 doses.	

TABLE 6-5. (continued)

Species	Route	Dose	Effect	Reference
Rat	Intraperitoneal	0.00955 and 0.01910 ml/kg in cottonseed oil daily for 30 days	Decreased weight gain, increased, kidney-to-body weight ratios, normal hematologic parameters, normal sodium sulfobromophthalein disappearance. Increased incidence of pulmonary lesions over controls. Other tissues normal.	Lawrence et al. (1973, 1974)
Rat	Intraperitoneal	0.04774 ml/kg in cottonseed oil 2 d/wk X 12 wk	Decreased food consumption, decreased body weight gain, decreased hemoglobin values and erythrocyte counts. Increased segmented neutrophils and decreased lymphocytes. Increased heart, kidney and liver-to-body weight ratio. No abnormal tissue pathology reported.	
Rat	Intraperitoneal	0.0190 ml/kg in cottonseed oil 3 d/wk X 12 wk	Decreased food consumption, decrease hemoglobin values and erythrocyte counts. Decreased lymphocytes. No abnormal tissue pathology reported.	
Rat	Intraperitoneal	0.0095 ml/kg in cottonseed oil 3 d/wk X 12 wk	Decreased hemoglobin values. No other findings.	

0.17 to 0.25 mg/l (170 to 250 mg/m^3). During the first month of exposure, these animals were not observably different from controls. In the following month, there was some deterioration in the condition of the animals. Hyperemia of the skin and subcutaneous hemorrhage were noted on some sites of the body, respiratory difficulty and dyspnea were apparent, and two animals died. There was some improvement after 2 months of exposure; however, in the fourth month there was a marked deterioration in the condition of the animals, and all remaining animals died at the start of the fifth month. The rats showed elevated blood pressure after 1-2 months of exposure. Microscopic examination of the tissues from these animals revealed changes in the lungs, kidneys, liver, and heart. Respiratory tract changes included bronchitis, with necrosis of the bronchial mucosa, thickened alveolar septa, and pulmonary edema. The kidneys showed deterioration and necrosis of the convoluted tubules. The hepatic cells showed some fatty degeneration and vacuolization. The staining of myocardial tissue was irregular, and there was fragmentation of the myocardial fibers.

The second group of rats was exposed to epichlorohydrin at a concentration of 0.02-0.06 mg/l (20-60 mg/m^3) for 3 hours a day for 6.5 months. No signs of toxicity were observed, and no deaths occurred during the study. The weight gain was 5-10 percent below that of the control animals. Two months after exposure began, blood pressures were slightly elevated (95-100 mmHg compared with 90-95 mmHg for the control animals). The changes observed in the tissues were similar in nature but not as severe as those observed at the higher exposure level.

Fomin (1966) exposed three groups of 15 male white rats (strain and weight unspecified) for 98 days (14 weeks) to epichlorohydrin at concentrations of 0.2, 2.0, and 20 mg/m^3 (0.05, 1.06, and 5.28 ppm). A fourth group not exposed to epichlorohydrin served as controls. The author examined body weights, blood nucleic acids, erythrocytes, leukocytes, hemoglobin, and urinary coproporphyrin. In addition, leukocytes were examined microscopically for their ability to fix a fluorescent dye (dye unspecified). Neurological/behavioral measurements were also made and are described in Section 6.3.

Nucleic acids in the blood decreased in concentration in the high exposure group beginning the second month of treatment and in the middle exposure group beginning the third month of treatment. The low exposure group and the control group showed no significant changes in nucleic acid levels during the study. After a recovery period of 4 weeks, the nucleic acid levels in the two higher exposure groups returned to normal. None of the rats exposed to epichlorohydrin had significant changes in erythrocyte, leukocyte, or hemoglobin levels. There was a dose-related increase in the number of leukocytes fixing a fluorescent dye, however, it was not made clear by the authors whether this could be interpreted as a meaningful toxic response. The necropsy microscopic examination of the tissues from the animals at the highest exposure level (20 mg/m^3) revealed emphysema, desquamative interstitial pneumonia, areas of edema, and deterioration of the connective tissue surrounding the blood vessels in the lung. There were intermuscular, microfocal hemorrhages and venous plethora (red florid complexion) in the heart, and necrotic changes in the convoluted tubules of the kidneys. Damage to the neurons in the medulla oblongata, cerebellum, and the hippocampus was also reported; however, these changes were not described in detail. Animals exposed to the lower levels had normal pathology.

Freuder and Leake (1941) exposed 10 white mice (strain, sex, and age unspecified) to epichlorohydrin at a concentration of 0.1 mM/l (2,370 ppm) daily for 1 hour. Exposures were continued until all animals were dead. Mortality was recorded daily. Table 6-6 shows the results of the study.

Table 6-6. Mortality in Mice Exposed to 2,500 ppm Epichlorohydrin

No. of Exposures	No. of Survivors
1-2	10/10
3-5	8/10
6	6/10
7	5/10
8	3/10
9-15	2/10
16	0/10

Source: Freuder and Leake (1941).

During the first two exposures, the only abnormal signs were irritation of the nose and eyes. The animals then showed decreased activity and muscular relaxation of the extremities, and respiration was depressed and increasingly difficult. Some animals experienced clonic convulsions.

The subchronic inhalation toxicity of epichlorohydrin was examined in two strains of rats (Fischer 344 and Sprague-Dawley) and one strain of mouse (B6C3F1) following repeated daily exposures (Quast et al. 1979a). Inhalation exposures were at 0, 5, 25, and 50 ppm (0, 19, 95, and 190 mg/m^3) of 99.8 percent epichlorohydrin for 6 hours/day, 5 days/week for a total of 61 or 62 exposures in 87 or 88 days for male and female animals, respectively. For each species and strain, 20 males and 20 females were used in each group; rats were 9-11 weeks old and mice were 7-9 weeks old at the start of inhalation exposure. An interim sacrifice of 10 animals of each sex per exposure group was made for each species and strain after 30 days of exposure, and histopathologic examinations were conducted on five animals of each sex of both the control and 50-ppm groups. After 90 days all surviving animals were killed. Clinical studies conducted on animals killed at 30 and 90 days included urinalysis (rats only), hematology, blood urea nitrogen, serum glucose concentrations, and serum enzyme activities glutamic-pyruvic transaminase (SGPT), glutamic-oxaloacetic transaminase (SGOT), and alkaline phosphatase (AP). Control animals and the 50-ppm exposed group were necropsied and the following organs were weighed and prepared for histopathologic examination: brain, heart, liver, kidneys, testes, spleen, and thymus. In addition, all possible target organs from the 5- and 25-ppm exposure group animals were microscopically examined at the 90-day sacrifice.

Inhalation of 5 ppm of epichlorohydrin (Quast et al. 1979a) did not result in toxicologically significant effects in rats or mice as determined by clinical observations or changes in body weight, hematology, urinalysis, clinical chemistry, organ weights, gross pathology, or histopathology.

During exposure, rats showed a dose-related conjunctival redness and eyelid spasms without evidence of ocular involvement. These effects appeared to be transient with recovery occurring overnight. Comparable observations were not made in mice simultaneously exposed with these rats. During the first 10 days of exposure, reduced activity was noted in the rats exposed to epichlorohydrin at 25 and 50 ppm.

There were no significant alterations in hematology, urinalysis or clinical chemistry parameters in any of the test animals. There was a slight decrease in body weight gain in male rats of both strains and in male and female mice at 50 ppm, and in female Fischer 344 rats at 25 ppm.

The most severely affected tissues in both rats and mice were the nasal cavities. There were inflammatory and degenerative changes in the olfactory and respiratory epithelia of animals exposed to 25 or 50 ppm of epichlorohydrin. The severity of lesions was dose related. Male rats were more severely affected than females, and histologic changes were more severe in Sprague-Dawley rats than in Fischer 344 rats. Mice were less severely affected than either strain of rat, and there was no apparent difference in severity of lesions between male and female mice.

Histopathologic changes were observed in the kidneys of rats of both strains exposed to 50 ppm epichlorohydrin, consisting of increased incidence of dilated tubules, focal tubular nephrosis and swelling of epithelial cells of the renal cortex. The severity of lesions of the kidney did not differ at 30-day or 90-day sacrifice, suggesting a lack of progression of the effect on repeated exposure. At 25 ppm, there were no histopathologic changes in rat kidneys; one female Sprague-Dawley rat had a unilateral kidney tumor that was not considered exposure related. There were no histopathologic changes in the kidneys of mice exposed to epichlorohydrin.

The livers of rats exposed to 50 ppm epichlorohydrin showed decreased glycogen deposits but no other histopathologic changes. A similar effect was not seen in mice.

At final sacrifice the adrenal glands of some male rats exposed to 50 ppm epichlorohydrin showed slight microvacuolation of cells in the zona fasciculata; this was possibly a stress response. In addition, in the epididymis of several male Sprague-Dawley rats from the 50-ppm group, there were increased numbers of nucleated cells and/or amorphous eosinophilic staining material within the lumen, although there was a normal sperm count.

In summary, the results of this study indicated that both rats and mice exposed to 25 or 50 ppm epichlorohydrin consistently had substantial changes in the epithelium of the nasal turbinates. Lesser effects in other tissues also occurred at these exposure levels, but with some variation in response. Rats and mice exposed to 5 ppm epichlorohydrin had no adverse effects in any of the parameters monitored in these studies.

To characterize target organ effects and to evaluate early changes in the nasal turbinates, Quast et al. (1979b), in a subsequent experiment, exposed Fischer 344 rats, Sprague-Dawley rats, and B6C3F1 mice to 100 ppm (380 mg/m^3) epichlorohydrin for 6 hours/day for 5 days during 1 week, and for 4 days during a 2nd week for a total of nine exposures in 12 days. Exposures were carried out in a 4.3-m^3 stainless steel and glass Rochester-type dynamic flow inhalation chamber. Five animals of either sex were used as test animals and controls; rats were 9 to 12 weeks old and mice were 7 weeks old at the start of the exposure.

When groups of animals were placed in the exposure chamber they huddled together and slept. No evidence of eye or nasal irritation was detected during exposure; however, upon removing the rats from the chamber following exposure, there was a slight amount of moist nasal discharge and discoloration of the hair immediately around the nasal orifice, suggestive of exudative rhinitis. This was not noticeable in the mice due to the dark hair and skin color of the species used. Immediately after the animals were removed from the exposure chamber they sneezed and rubbed their noses. Signs of respiratory distress, apparent decreased food intake (actual food consumption not measured), and reduced fecal excretion were observed during exposure periods with some recovery observed on the weekends.

A marked decrease in the body weight of rats and mice was observed during the exposure to 100 ppm (380 mg/m^3) epichlorohydrin and equally apparent was a transient partial recovery of body weight following the weekend.

After nine exposures (day 12), all animals were killed. Weights of brain, heart, liver, kidneys, and spleen were recorded. Samples of blood and urine were collected on day 11 for hematology and urinalysis evaluations. On day 12, at time of necropsy, blood was obtained from rats to determine the serum concentrations of urea nitrogen, glucose, and the glutamic pyruvic transaminase (SGPT), glutamic-oxaloacetic transaminase (SGOT), and alkaline phosphatase (AP) activities.

Multiple effects were associated with repeated exposures to 100 ppm epichlorohydrin. The most consistently recognized treatment-related effects were changes in the mucosa of the nasal turbinates, decreased body weight gain, leukocytosis secondary to nasal inflammation, decreased specific gravity of urine (hematologic and urinary examination were conducted only on rats), and increased kidney weights in rats, but not mice.

Upon histopathologic examination of tissues, the most consistent and readily detectable changes were present in the nasal turbinates, with degeneration, inflammation, hyperplasia, and squamous metaplasia present to some degree in all exposed rats and mice. This condition extended throughout the regions lined by respiratory and olfactory epithelium. These changes were more severe than those noted in the rats and mice exposed to 25 and 50 ppm epichlorohydrin in the 90-day subchronic study by Quast et al. (1979a). Changes in the respiratory tract of mice were much less severe than those in either species of rat but were similar in nature. Occasional sections of rat trachea had an increased number of inflammatory cells migrating through the epithelial lining. Dose-related changes in the liver were minimal for both animals. These changes were characterized by decreased hepatocellular glycogen content, decreased hepatocyte size, and increased variability in cytoplasmic staining.

Degenerative changes were noted in the kidneys of both strains of rats, but not the mice. Minor nondegenerative liver effects and thymic atrophy, both secondary to stress, were noted in rats and mice. Male rats of both strains had slight changes in the contents of the epididymides. Male Sprague-Dawley rats had minor changes in the adrenal glands, possibly secondary to stress. In general, there was a decreasing order of toxicity observed as follows: Sprague-Dawley rats, Fischer 344 rats, and B6C3F1 mice.

Laskin et al. (1980) examined the chronic toxicity of epichlorohydrin in rats. Two groups of 100 male Sprague-Dawley rats were exposed by inhalation to epichlorohydrin concentrations of 10 and 30 ppm (37.8 and 114 mg/m^3). The animals were exposed 6 hours/day, 5 days/week over their lifetimes. An additional group of 140 rats was exposed to 100 ppm epichlorohydrin for thirty 6-hour exposures and then observed over their lifetimes. A sham control group of 100 rats was exposed to air alone in an exposure chamber using the lifetime exposure schedule. Finally, a group of 50 rats was maintained as untreated controls. The laboratory methods for inhalation exposure, necropsy, preparation of tissues, and histopathologic observations are reported in Section 7.1 (Carcinogenicity).

The weight gain for the animals exposed to 10 ppm epichlorohydrin was comparable to that in controls, but the group exposed to 30 ppm began to show marked decreases in body weight after 40 weeks. For the first 16 weeks of the study, there was no significant mortality in either exposure group. However, by 48 weeks, 45 percent of the group exposed to 10 ppm had died and by 60 weeks a similar number of the group exposed to 30 ppm had died. In all cases, pulmonary congestion and pneumonia were observed. Mortality was not treatment related; in fact, a slightly greater mortality rate was noted in control groups of rats than in treated groups.

At necropsy, renal damage was observed at a high incidence in epichlorohydrin-treated animals. The severities of the lesions were similar for the animals treated at 30 and 10 ppm. The incidence of kidney lesions was 65, 37, 24, and 14 percent for the 30-ppm, 10-ppm, sham controls, and untreated controls, respectively. Tubular degenerative changes were the most common lesions. The tubules were atrophied, dilated, and some were filled with hyaline casts. Occasionally, atrophy of the glomeruli was also observed.

The authors observed a high incidence of rhinitis and pulmonary infection in both control groups. Approximately 90 percent of the control animals showed severe inflammatory changes in the nasal cavity. For this reason, the authors could not attribute effects observed in the nasal cavity of exposed animals to epichlorohydrin exposure. However, none of the control animals showed squamous metaplasia of the nasal mucosa.

6.1.2.2 Oral--Freuder and Leake (1941) examined the oral toxicity in mice administered repeated doses of epichlorohydrin by gavage. Three groups of 15 white mice (strain, sex, and weight unspecified) received 10 mg/kg epichlorohydrin suspended in a 25 percent aqueous gum arabic. Single daily doses were given until all animals died. The number of doses required to produce the first death and 100 percent mortality are shown in Table 6-7.

Table 6-7. Mortality of Mice Administered Epichlorohydrin Orally

Dose (mg/kg)	First Death (day)	100% Mortality (day)
94	2	21
190	1	8
271	1	4

Source: Freuder and Leake (1941).

Repeated oral administration produced many of the same signs described for inhalation exposure: decreased activity, muscular relaxation of the extremities, stiffening of the tail, fine tremors, depressed respiration, and clonic convulsions.

6.1.2.3 Intraperitoneal Injection--Lawrence et al. (1972, 1974) examined the cumulative toxicity of epichlorohydrin by repeated intraperitoneal injection in rats. Two groups of 12 male Sprague-Dawley rats, weighing 100-150 g received 30 daily injections of 0.00955 and 0.01910 ml/kg epichlorohydrin dissolved in cottonseed oil. A control group received injections of cottonseed oil alone for the 30-day period. The rats were weighed at 5-day intervals throughout the study. Clinical blood chemistry, rate of clearance of sodium sulfobromophthalein from plasma, organ-to-body weight ratios, and organ pathology were examined at the end of the 30-day exposure period. No deaths occurred during the treatment period. Weight gain was significantly less ($p \leq 0.05$) in the low dose group than in the control group beginning on day 20 and in the high dose group on day 15. Impaired hepatic function was not detected by the rate of clearance of sodium sulfobromophthalein. There were no marked differences in the hematologic data between dosed and control animals. However, kidney-to-body weight ratios were significantly increased ($p \geq 0.05$) in both epichlorohydrin-dosed groups. Microscopic examination of the tissues showed a greater incidence and severity of lesions in the lungs of the dosed animals than in control animals; however, the control group as well as the test groups showed pulmonary changes such as bronchitis, peribronchitis, interstitial pneumonia, bronchopneumonia, and emphysema. Microscopic examination of the other organs did not reveal any significant changes.

Lawrence et al. (1972, 1974) also examined the cumulative toxicity of epichlorohydrin by repeated intraperitoneal injection in rats, but used a different dosing schedule. In this study, three groups of 12 immature, male, Sprague-Dawley rats weighing 60 to 100 g received repeated injections of 0.0095, 0.0190, or 0.04774 ml/kg epichlorohydrin in cottonseed oil 3 days per week for 12 weeks. A fourth control group received injections of cottonseed oil alone according to the same schedule. Food consumption (weeks 1, 7, and 12) and weekly body weights were monitored throughout the study and clinical blood chemistry, organ-to-body weight ratios, and organ pathology were examined at the end of the 12th week of the study. Food consumption was generally lower for the two high dose groups than for the controls. Body weight gain was significantly lower ($p \geqq 0.05$) for the high dose group each week of the study, except weeks 2, 3, and 12. The hematological studies showed a dose-related decrease in hemoglobin, hematocrit values, and erythrocyte counts in the epichlorohydrin-treated animals. Hemoglobin concentration was significantly ($p \geqq 0.05$) decreased at all three dose levels and the hematocrit value was significantly ($p = 0.05$) decreased only at the middle dose level. An increase in segmented neutrophils was observed at the high dose level, and reductions in the percentage of lymphocytes were observed in the two higher dose groups. Organ-to-body weight ratios were not significantly different in the low and middle dose groups; however, in the high dose group, significant ($p \geqq 0.05$) increases were observed for the heart, kidneys, liver, and brain. No abnormal organ pathology was reported.

6.1.2.4 Dermal--Freuder and Leake (1941) examined toxicity associated with the repeated dermal exposure of rats to epichlorohydrin. Undiluted epichlorohydrin was placed on a square centimeter piece of gauze and applied to the shaved skin on the abdomen of 10 rats (strain, sex, and age unspecified). The gauze was removed after 1 hour; the application was repeated daily. Two dose levels were examined, 6.5 mmol/kg and 13.0 mmol/kg (0.5 ml/kg and 1.0 ml/kg). Mortality data are shown in Table 6-8. Repeated applications caused superficial necroses of the skin that exceeded the size of the gauze. The skin was parched and brown. In the rats that survived, the skin gradually healed with no other symptoms of toxicity noted.

Table 6-8. Lethality Following Repeated Dermal Application of Epichlorohydrin in Rats

Dose (ml/kg)	No. of Applications	No. of Survivors
0.5	1	10/10
	2	6/10
	3	4/10
	4	0/10
1.0	1	8/10
	2	6/10
	3	6/10

Source: Freuder and Leake (1941).

6.2 EFFECTS ON THE LIVER, KIDNEYS, AND LUNGS

Toxic effects in the kidneys and possibly in the liver and lungs have been reported following exposure to epichlorohydrin, and have been discussed in Section 6.1; the following is a summary of these effects.

6.2.1 Liver

Inhalation exposure of rats (170-250 mg/m^3, 3 hours/day for 5 months) caused a moderate degree of fatty degeneration and vacuolization of hepatic cells (Kremneva and Tolgskaya 1961). Exposure of rats to 190 mg/m^3 epichlorohydrin, 6 hours/day, 5 days/week for 61 exposures caused a decreased hepatocellular glycogen content (Quast et al. 1979a).

Gage (1959) also found that nineteen 6-hour exposures to 120 ppm (456 mg/m^3) epichlorohydrin caused congestion of the liver of rats. Lawrence and Autian (1972) found that exposure of ICR mice to epichlorohydrin caused a dose-related increase in phenobarbital-induced sleep time. This assay indicates inhibition of liver microsomal enzymes. When microsomal enzymes are inhibited, sleep time is prolonged. Four groups of 10 mice each were exposed to 98.20 mg/l epichlorohydrin for 0.1, 0.2. and 0.5 of the LT_{50} (0.92, 1.83, and 4.58 minutes, respectively). A control group was placed in the exposure chamber for 4.58 minutes and exposed to air. Increased sleep times were observed for mice exposed to increasing concentrations of epichlorohydrin. The sleep time for the control mice was 69.20 ± 3.88 minutes. For those exposed for 0.92 minutes, the sleep time was 73.14 ± 6.46 minutes; for those exposed for 1.83 minutes, sleep time was 85.31 ± 6.40 minutes; and for those exposed for 4.58 minutes, sleep time was 108.93 ± 9.34 minutes. This dose-related increase indicates inhibition of the liver microsomal enzyme system.

6.2.2 Kidneys

Epichlorohydrin exposure has been shown by several investigations to cause severe renal toxicity by different routes of exposure (Table 6-5). The most extensive changes observed were in the convoluted tubules (Kremneva and Tolgskaya 1961, Rotaru and Pallade 1966, Laskin et al. 1980). The earliest changes were swollen, dilated, and ischemic convoluted tubules. This was followed by epithelial degeneration. Later, the epithelia became completely necrotic, and cells were desquamated into the lumen of the tubules where they underwent calcification. After exposure ceased, regeneration of the tubular epithelium occurred. Rotaru and Pallade (1966) and Pallade et al. (1967) found signs of regeneration in the kidneys in rats 5 days after subcutaneous injection of epichlorohydrin. Ten days after exposure the investigators found marked regeneration of the tissue in the kidneys with most of the tubular integrity restored. In addition to changes in tubules, several authors (Kremneva and Tolgskaya 1961; Rotaru and Pallade 1966; Gage 1959; Laskin et al. 1980) have reported minor glomerular changes in rodent kidneys following epichlorohydrin exposure.

6.2.3 Lungs

Carpenter et al. (1949) reported acute respiratory irritation, hemorrhage, and severe pulmonary edema in rats exposed to epichlorohydrin by inhalation at concentrations ranging from 283 to 445 ppm (1,075 to 1,691 mg/m^3) for 4 hours. There were also marked increases in the lung-to-body weight ratios for animals exposed at higher concentrations. At 369 ppm (1,042 mg/m^3)

epichlorohydrin, an 80 percent increase in lung-to-body weight ratio was observed when compared with controls. The two lowest exposure levels (283 and 303 ppm) showed no lung-to-body weight ratio increases over controls.

Quast et al. (1979a) found that inhalation of 50 ppm epichlorohydrin (6 hours day, 5 days/week for 88 days) caused focal subacute pneumonitis in mice, but not in rats.

Changes in the lungs have also been observed following epichlorohydrin exposure by routes other than inhalation. Rotaru and Pallade (1966) and Pallade et al. (1967) described the pulmonary changes in rats following a single subcutaneous injection of either 150 or 180 mg/kg of epichlorohydrin. There was inflammatory desquamative bronchitis, edema of the bronchio-vascular connective tissue, and congestion of the alveolar septa.

6.3 BEHAVIORAL TOXICITY AND CENTRAL NERVOUS SYSTEM EFFECTS

Depression of the central nervous system (CNS) has been linked with acute exposure to high levels of epichlorohydrin. A range of 1,416 to 2,124 ppm epichlorohydrin was found to be lethal in rats during a 2-hour exposure (Kremneva 1960). These animals first became quiescent and then developed cyanosis and muscular relaxation of the extremities. This was followed by tail stiffening and fine tremor of the body; the respiration became increasingly depressed and some animals experienced clonic convulsions. Death occurred from depression of the respiratory center. This follows the common clinical development of toxicity from high acute exposures to epichlorohydrin.

Freuder and Leake (1941) exposed white mice (sex, age, and strain unspecified) by inhalation to epichlorohydrin at concentrations of 2,370 ppm (9,000 mg/m^3) for 60 minutes and at 8,300 and 16,600 ppm (31,540 and 63,080 mg/m^3) for 30 minutes. Within 24 hours after exposure, all the animals that were exposed at 8,300 and 16,600 ppm died. Delirium was observed 3 minutes after exposure started at 16,600 ppm and within 14 minutes after exposure started at 8,300 ppm. This was followed by the progressive depression of the CNS as previously described.

Fomin (1966) measured the latency time for defensive unconditioned reflex reactions in rats exposed to epichlorohydrin. Three groups, each containing 15 white male rats (strain and weight unspecified), were exposed by inhalation to epichlorohydrin at concentrations of 0.2, 2.0, and 20.0 mg/m^3 (0.05, 0.52, and 5.3 ppm). The animals were exposed continuously for 98 days. A fourth group, not exposed to epichlorohydrin, served as a control.The latency time was measured weekly using a technique described by Gusev and Minayev (1973). In this technique, the rat was placed in a chamber with a floor containing parallel metal bars or plates on which its extremities rested. The lid of the chamber contained a switch that rested on the back of the animal. Sufficient current was used in the parallel metal plates to startle the rat, causing the switch on the back of the animal to break the circuit. The time that elapsed between the application of the electrical stimulus and the rat breaking the circuit was the latency time for the defensive unconditioned reflex reaction.

The rats in the highest exposure group were hyperactive and restless on the 1st day of exposure. This was replaced by depression and decreased activity as exposure continued. After 1.5 months of exposure at 20.0 mg/m^3, the latency time for defensive unconditioned reflex reaction increased significantly in the exposed animals. The groups exposed at 0.2 and 2.0 mg/m^3 epichlorohydrin had latency times similar to the control group. Microscopic examination of the tissues and organs of the rats exposed at 20 mg/m^3 showed abnormal changes in the lungs and kidneys similar to those already described. The authors also observed damage to the neurons in the medulla oblongata, cerebellum, and hippocampus. These changes were not described in detail. No differences were observed between the animals exposed at 0.2 and 2.0 mg/m^3 and the control animals.

Kremneva and Tolgskaya (1961) examined the effects of prolonged epichlorohydrin exposure on the CNS of two groups of rats. The first group of eight rats (strain, sex, and age unspecified) were exposed daily (exposure period unspecified; probably 2 hours/day) for 5 months to epichlorohydrin vapor at 0.17-0.25 mg/l (50-60 ppm). The threshold of irritation (stimulation threshold) was measured in these experimental animals at various intervals throughout exposure. The stimulation threshold was measured by determining the amount of current required to elicit a withdrawal response in the animals. No further experimental details were provided. The animals exposed to epichlorohydrin at 0.17-0.25 mg/l showed an increase in stimulation threshold (8 mA to approximately 9 mA) during the first 2 months of exposure. At approximately 3 months, the threshold in the treated animals decreased to 8.5 mA and then by the 4th month it had increased again to approximately 9 mA. The control animals remained at a threshold of approximately 8 mA throughout the study. In this study, two rats died after 1.5 months of exposure. The remaining animals died at the start of the 5th month of exposure. The second group of 10 rats (strain, sex, and age unspecified) were exposed to epichlorohydrin vapors at a concentration of 0.02 to 0.06 mg/l (5-16 ppm), 3 hours/day for 6.5 months. The stimulation threshold was also measured in these animals. The threshold was higher in the animals exposed to epichlorohydrin from months 2 through 5. This threshold was 7.5-8.0 mA compared with 6.5-7.0 mA for the control animals. Six months into the study the stimulation threshold in the epichlorohydrin-treated animals decreased to approximately normal levels. No animals died at this exposure level during 6.5 months of exposure.

6.4 OTHER TISSUES OR ORGANS

6.4.1 Nasal Cavity

Irritation of the mucous membranes of the upper respiratory tract was a common finding in laboratory animals exposed to epichlorohydrin vapor. Irritation was normally followed by rhinitis (inflammation of the nasal cavities) and degeneration and necrosis of the nasal mucosa (Quast et al. 1979a). These effects have been described in detail in Section 6.1.2.1. Laskin et al. (1980) reported the development of neoplastic lesions of the nasal cavity in rats during chronic inhalation studies.

6.4.2 Eyes

Lawrence et al. (1972) instilled 0.1 ml of different concentrations of epichlorohydrin in cottonseed oil into the superior temporal quadrant of a rabbit's right eye; the left eye served as the untreated control. The eyes were then examined every 30 minutes for 3 hours and scored for the degree of irritation. No irritation was observed at 5 percent, and doubtful irritation was observed at 10 percent epichlorohydrin. However, an epichlorohydrin concentration of 20 percent produced conjunctival and palpebral irritation with edema; a 40 percent concentration produced iritis and palpebral irritation with edema; and an 80 percent epichlorohydrin solution produced corneal injury.

Smyth and Carpenter (1948) described corneal injury of grade 4 to the rabbit's eye (experimental methods not described) after epichlorohydrin instillation. The amount of test compound instilled was not stated. Grade 4 was described as moderately severe corneal injury.

Kremnera and Tolgskaya (1961) instilled a single drop of epichlorohydrin into the conjunctival sac of a rabbit's eye. The epichlorohydrin produced blepharospasm, hypermia of the mucosa, excessive lacrimation, papillary constriction, and corneal clouding. The corneal clouding had cleared after 2-4 days and improvement in the condition of the eye was noted. Complete recovery was reported within 7-10 days following exposure.

6.4.3 Circulatory System

Kremneva and Tolgskaya (1961) found elevated blood pressure and some pathologic changes in the myocardium (heart muscle) in rats exposed to epichlorohydrin at 170-250 mg/m^3 for 3 hours/day for 5 months. These changes were described as moderate. No other reports of cardiotoxicity have been found in the literature.

6.5 SUMMARY

Acute exposure to high levels of epichlorohydrin was shown to cause CNS depression and death resulting from respiratory paralysis. The LC_{50} in rats was 360 ppm (1,368 mg/m^3) for 6 hours, and the no-observed-effect-level (NOEL) was 283 ppm (1,075 mg/m^3) epichlorohydrin for 6 hours. With shorter periods of exposure, there were higher LC_{50} values. These LC_{50} values were similar when rats and mice were analogously exposed. Intraperitoneal LD_{50}s were found at 187 mg/kg for rats and 165 mg/kg for mice; and oral LD_{50}s for rats and mice were 248 and 236 mg/kg, respectively. In addition, epichlorohydrin was acutely toxic by the dermal route; a single immersion of a mouse's tail for 1 hour caused 100 percent mortality, and 0.64-1.3 ml/kg was the dermal LD_{50} in rabbits. A single nonlethal dose of epichlorohydrin can cause kidney and lung damage in rats.

Repeated exposures to epichlorohydrin were found to be highly irritating to the nasal cavity and to produce damage of the nasal mucosa in rodents. Subchronic exposure to epichlorohydrin has been shown to cause severe renal toxicity in rats via different routes of administration.

By both the inhalation and subcutaneous routes, epichlorohydrin has been shown to cause changes in the lungs and bronchi. Subchronic exposure of rats to toxic levels of epichlorohydrin caused mild effects on the liver and moderate changes in the myocardium. Epichlorohydrin has been found irritating to the skin and eyes of rabbits.

7. CARCINOGENICITY, MUTAGENICITY, AND REPRODUCTIVE AND TERATOGENIC EFFECTS

7.1 CARCINOGENICITY

7.1.1 Introduction

The purpose of this section is to provide an evaluation of the likelihood that epichlorohydrin is a human carcinogen, and, on the assumption that it is a human carcinogen, to provide a basis for estimating its public health impact, including a potency evaluation in relation to other carcinogens. The evaluation of carcinogenicity depends in part on available animal bioassays and epidemiologic evidence. Additionally, information on mutagenicity and metabolism (reviewed in other sections of this document) particularly in relation to chemical interaction with DNA and pharmacokinetic behavior, has an important bearing on qualitative and quantitative assessments of carcinogenicity. This section presents evaluations of the animal bioassays, each of which are followed by a qualitative statement as to the evidence of carcinogenicity and its relevance to quantitative human risk assessment. This section also evaluates the epidemiologic evidence. The epidemiologic and animal studies are used, where appropriate, for human quantitative risk assessment. Lastly, a summary and conclusions are presented dealing with relevant aspects of the carcinogenicity of epichlorohydrin.

7.1.2 Animal Studies

In separate studies, epichlorohydrin exposure produced carcinogenic responses proximal to the site of exposure. The results of these studies were as follows: anterior nasal cavity squamous cell carcinomas in an inhalation experiment, sarcomas at the site of subcutaneous injection, and papillomas and carcinomas in the forestomach in a drinking water study and in a gavage (in water) study. These carcinogenic responses were related to the amounts of epichlorohydrin to which the test animals were exposed.

7.1.2.1 Inhalation Exposure: Rat--Rats were exposed to epichlorohydrin vapor (Aldrich Chemical Company, $\geq$ 99 percent pure by gas chromatography, Laskin et al., 1980). Exposures were perfomed in 128-liter or 1.3-m^3 inhalation chambers. Ambient epichlorohydrin levels were monitored spectrophotometrically during exposures. Test animals were non-inbred male Sprague-Dawley rats initially 8 weeks old. Body weights were recorded monthly. Rats were allowed to live until natural death or were killed *in extremis*. Necropsies were performed, and tumors, lesions, and major organs were examined histopathologically. Heads were fixed, decalcified, and sectioned for examination of the entire nasal cavity.

Based on the results of preliminary LC_{50} studies, 100 ppm epichlorohydrin was selected as the exposure level for a 30-day exposure period. Initially, 40 rats were exposed to 30 daily exposures of 6 hours each; subsequently, after the exposure period for the 40 rats was completed, another group of 100 rats was also given 30 daily 6-hour exposures. Both groups were followed by lifetime observation.

Early mortality, attributed to respiratory disease, was higher in sham (exposed to air only) and untreated control groups as compared to treated animals (Figure 7-1). Body weight gain was comparable among groups. A maximum weight gain of 200-220 percent achieved by 48 weeks following the first exposure was sustained or slightly declined during the rest of the study.

Severe inflammatory changes in the respiratory tract were found in almost all treated animals as well as in 90 percent of the control animals. Edema, congestion, and pneumonia were observed in the lungs of exposed rats. Control rats had congestion, edema, bronchiectasis, and pneumonia in the lungs. Control rats had some expected kidney changes commonly found in aging rats. Renal damage, including dilatation of cortical and medullary tubules, was found in 63 percent of the treated rats.

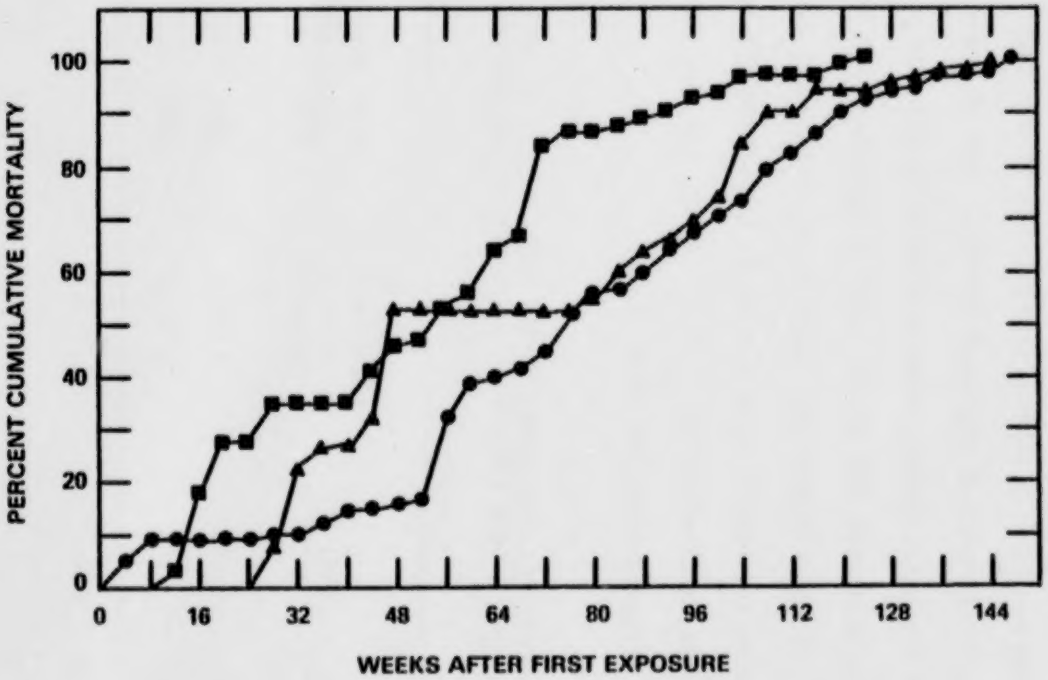


Figure 7-1. Mortality of rats following exposure to 100 ppm of epichlorohydrin (6 hr/day for 30 days). The curves represent air-treated controls (■), untreated controls (▲), and a group of 140 animals exposed to epichlorohydrin (●).

SOURCE: Laskin et al., 1980.

Squamous cell carcinomas were found in the nasal tracts of treated animals, as enumerated in Tables 7-1 and 7-2. Many of these nasal carcinomas infiltrated the proximal bones of the skull, but metastasis to distal organs was not found. The nasal carcinomas were described in terms of their histologic appearance as well-differentiated tumors with keratin pearls. All of the nasal carcinomas appeared in the anterior portion of the nasal cavity. Some of these solid tumors almost filled the nasal cavity, thereby causing dyspnea and wheezing. Three other rats were diagnosed with either nasal or bronchial and larynx papillomas. The incidence of other tumor types in nonrespiratory organs was similar between treated and control groups, thereby indicating the lack of epichlorohydrin-induced carcinogenicity in other organs, and the lack of metastasis from the proximal site, the nasal cavity.

TABLE 7-1. SQUAMOUS CELL CARCINOMAS OF THE NASAL CAVITY OF MALE WISTAR RATS FOLLOWING THIRTY 6-HOUR EXPOSURES TO 100 PPM EPICHLOROHYDRIN VAPOR

Experiment	Dose (ppm)	No. of animals	No. of tumor-bearing animals (percent)	Tumor observation time (days)	
				Mean	Range
1	100	40	4 (10)	540	462-610
2	100	100	11 (11)	623	330-933

SOURCE: Laskin et al., 1980.

A second study was done in which 100 male rats per treatment group were exposed to 10 or 30 ppm epichlorohydrin 6 hours/day, 5 days/week, for their lifetimes. Treated animals were compared with concurrent sham and untreated control groups.

TABLE 7-2. DOSE-RESPONSE FOR INDUCTION OF SQUAMOUS CELL CARCINOMAS IN THE NASAL CAVITY OF MALE WISTAR RATS EXPOSED TO EPICHLOROHYDRIN VAPOR

Concentration ppm	Number of exposures	Total exposure (ppm x days)	Cancer incidence (No. with cancer)/(No. exposed)
100 (combined studies)	30	3,000	15/140[bc]
30	290[a]	8,700	1/100
10	250[a]	2,500	0/100
Air (sham) controls for life	---	---	0/100
Untreated controls	---	---	0/50

[a] Lifetime exposures were based on median survival time.
[b] $p < 0.00001$ versus combined controls.
[c] There were also two nasal papillomas in this group.

SOURCE: Adapted from Laskin et al., 1980.

Early mortality was high in all groups, with 50 percent mortality evident by 64 weeks (Figure 7-2). Lung congestion and pneumonia were common in decedents. Body weights were lower in the 30-ppm group, as shown in Figure 7-3.

Two respiratory tract tumors were found: a larynx squamous papilloma (at 90 weeks) and a squamous cell carcinoma (at 107 weeks) in two rats exposed to 30 ppm. The Laskin et al. (1980) paper incorrectly described the larynx papilloma as a nasal papilloma. This was corrected in a telephone conversation with one of the authors. Severe inflammation of the nasal cavity was noted in 90 percent of the control animals. Exposure to 10 and 30 ppm epichlorohydrin produced 2 and 4 percent incidences, respectively, of squamous cell metaplasia in the nasal cavity, with none in the controls. Squamous cell metaplasia is the transformation of the nasal mucosal epithelium into stratified squamous epithelium.

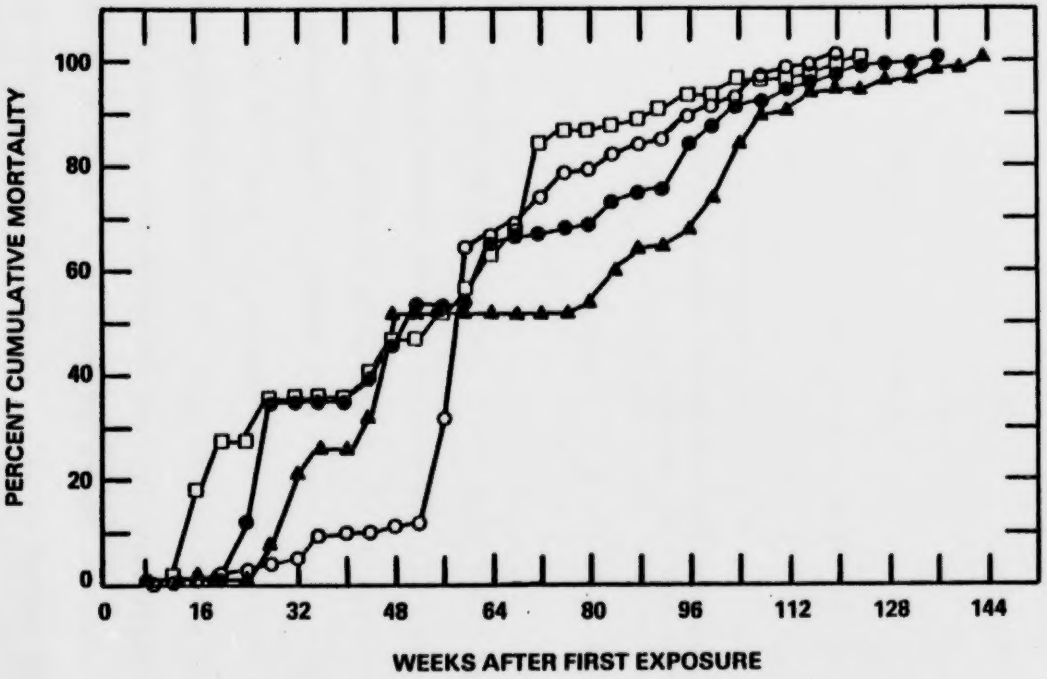


Figure 7-2. Mortality of rats following lifetime exposure (6 hr/day, 5 days/week) to epichlorohydrin. The curves represent air-treated controls (☐), untreated controls (▲), exposure to 30 ppm (○), and exposure to 10 ppm (●).

SOURCE: Laskin et al., 1980.

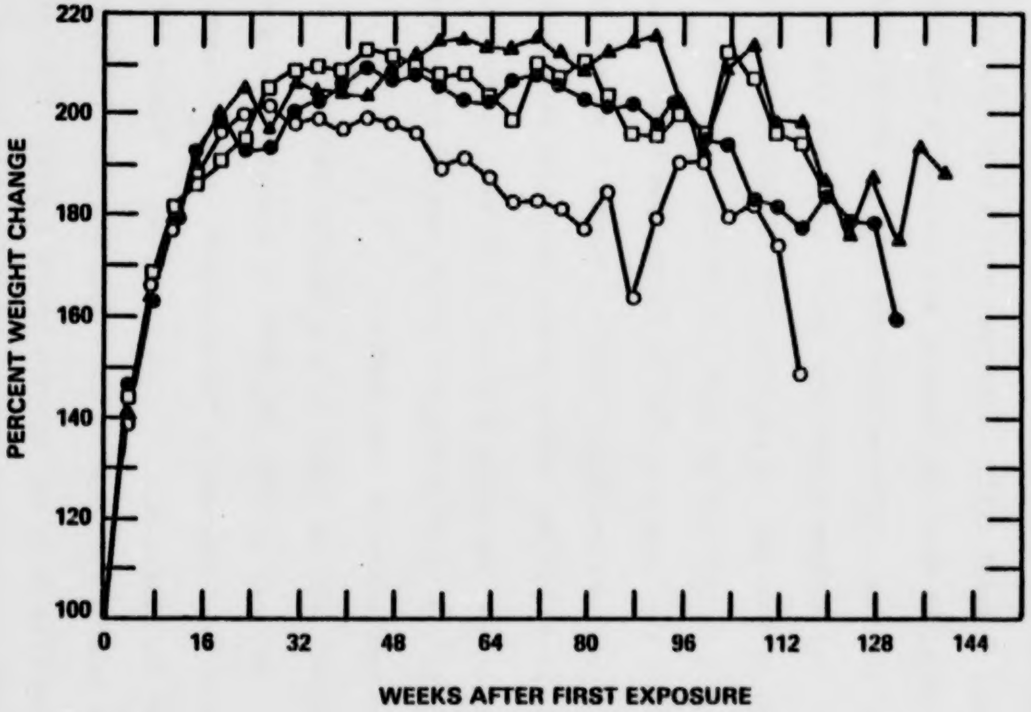


Figure 7-3. Growth of rats following chronic exposure to epichlorohydrin. The curves represent air-treated controls (□), untreated controls (▲), and animals exposed to 30 ppm (○) or 10 ppm (●) epichlorohydrin. Exposures were done for 6 hr/day, 5 days/week for the lifetimes of the animals.

SOURCE: Laskin et al., 1980.

Renal damage occurred in 65, 37, 24, and 17 percent of the 30 ppm, 10 ppm, sham, and untreated groups, respectively. Severity of renal damage, diagnosed as mainly tubular degenerative changes, was related to epichlorohydrin dose.

The results of the Laskin et al. (1980) study have provided qualitative evidence for the carcinogenicity of epichlorohydrin. Epichlorohydrin produced a much greater increase in nasal cavity carcinomas with a high dose given early in the study (15/140 plus two nasal papillomas), but produced a much lesser response (1/100) when given as a lifetime treatment with one-third of the higher concentration. The authors stated that nasal carcinomas had not been observed in 1,920 control rats over 14 years in their laboratory; however, no data were made available on the rate of nasal inflammation. Laskin et al. (1980) hypothesized that the latency period for cancer development could have been shorter with the more intense (though shorter) exposure, whereas in the lifetime study at 10 and 30 ppm the latency period could have been longer, perhaps approximating or even exceeding the rats' lifetime. Furthermore, the rather high mortality in the lifetime exposure groups reduced the number of animals available for development of late tumors.

Since epichlorohydrin was not found to be a complete carcinogen in a dermal study (Van Duuren et al., 1972a, b), there is a question as to whether nasal tumors would have been observed in the absence of nasal inflammation. One of the authors of the Laskin et al. (1980) study, Roy E. Albert, stated that inflammation in control rats used in their laboratory for lifetime inhalation carcinogenicity studies is not apparent before 1 year. Since exposure to the carcinogenic 100-ppm exposure level of epichlorohydrin occurred during the initial 30 exposure days (at age 8 weeks) of the Laskin et al. (1980) studies, the probability that nasal inflammation as observed in matched controls could have been an initiating event in the induction of nasal carcinomas is considered to be low because inflammation was not observed during the early or initiating-event period. It has generally been observed that most promoters are irritants, but that not all irritants are promoters. There is presently no evidence available to suggest a promoting action of epichlorohydrin. Further, it has not been determined that the observed nasal inflammation necessarily caused the induction of the observed nasal carcinomas (personal communication with R.E. Albert).

The Laskin et al. study indicates qualitatively that epichlorohydrin is an animal carcinogen. The 30x-exposure study showed a significant increase in a rare tumor type, nasal carcinomas, 15/40 vs. controls ($P < 0.0001$), whereas the 30-ppm response in the lifetime exposure response (1/100) was not significant compared to internal controls ($P > 0.05$) but was significant when compared to external control rat nasal carcinomas (0/1920) observed in the authors' laboratory over 14 years ($P = 0.05$). On these bases, the Laskin et al. study can be used to extrapolate a 95% upper-bound risk estimate for humans.

7.1.2.2 Oral Administration in Drinking Water: Rat--Konishi et al. (1980) and Kawabata (1981), both reporting on the same study, described a carcinogenicity bioassay on epichlorohydrin given orally in drinking water to rats. The epichlorohydrin (Hani Kagaku, Kyoto) was 99.96 percent pure; impurities, if known, were not reported. Seventy-two male outbred Wistar rats, 6 weeks old and each weighing 160 g, were divided into four groups of 18 rats each. Six animals were housed in each cage. The animals were given fresh solutions of epichlorohydrin in drinking water each day. The epichlorohydrin solutions were protected from light. One group served as untreated controls, and the other three groups were treated with 375, 750, or 1,500 ppm epichlorohydrin. These concentrations of epichlorohydrin in water (ppm) were initially 0, 62, 95.5, and 187 mg/kg body weight/day (approx.). The amount of epichlorohydrin ingested per rat was constant throughout the experiment (because daily water consumption was constant), and thus, the dosage rate (mg epichlorohydrin/kg/day) varied inversely with body weight. The total epichlorohydrin dosage for the whole experiment was 0, 5.0, 8.9 and 15.1 grams for each of the groups. Although survival among all groups was similar, treatment with epichlorohydrin was discontinued for short periods after 60 weeks due to debilitation of the rats (Figure 7-4). It was concluded that pulmonary infection was the cause of death in animals that died during the course of the experiment. Epichlorohydrin intake patterns during the 81-week experimental period are shown in Figure 7-5.

All of the animals were necropsied at 81 weeks terminal sacrifice, and tissues and organs, as well as tumors, were examined histopathologically. However, the authors did not report pathologic results for animals that died during the study. Major organs from survivors were weighed, and blood was collected from survivors for biochemical and hematologic analysis.

Dose-related decreases in body weight gain occurred as shown in Figure 7-6. Statistically significant ($P < 0.05$) increases in organ/body weight ratios were common in the groups given 750 or 1,500 ppm epichlorohydrin, largely due to comparable organ weights among control and treatment groups but decreased body weights in treated animals. A significant ($P < 0.05$) increase in pancreas/body weight ratios was also evident in the 375-ppm group. The results of the pathologic examination of the kidneys were not reported; however, significant ($P < 0.05$) increases in both kidney weights and kidney/body weight ratios in each treatment group (Table 7-3) may be indicative of injury at this organ site from treatment with epichlorohydrin, since treatment-related kidney damage was also seen in the Laskin et al. (1980) study, in which exposure was by inhalation.

The results of blood analyses were normal except for significant ($P < 0.05$) increases in cholesterol and neutral lipid levels in each treatment group as

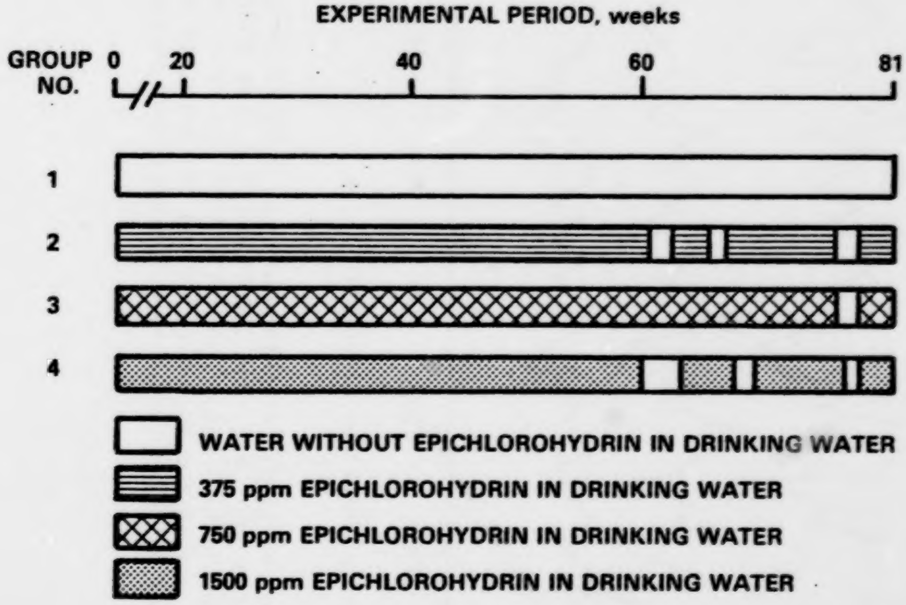

EXPERIMENTAL PERIOD, weeks
GROUP NO.
0
20
40
60
81
1
2
3
4
WATER WITHOUT EPICHLOROHYDRIN IN DRINKING WATER
375 ppm EPICHLOROHYDRIN IN DRINKING WATER
750 ppm EPICHLOROHYDRIN IN DRINKING WATER
1500 ppm EPICHLOROHYDRIN IN DRINKING WATER

Figure 7-4. Patterns of epichlorohydrin administration in male Wistar rats.

SOURCE: Konishi et al., 1980.

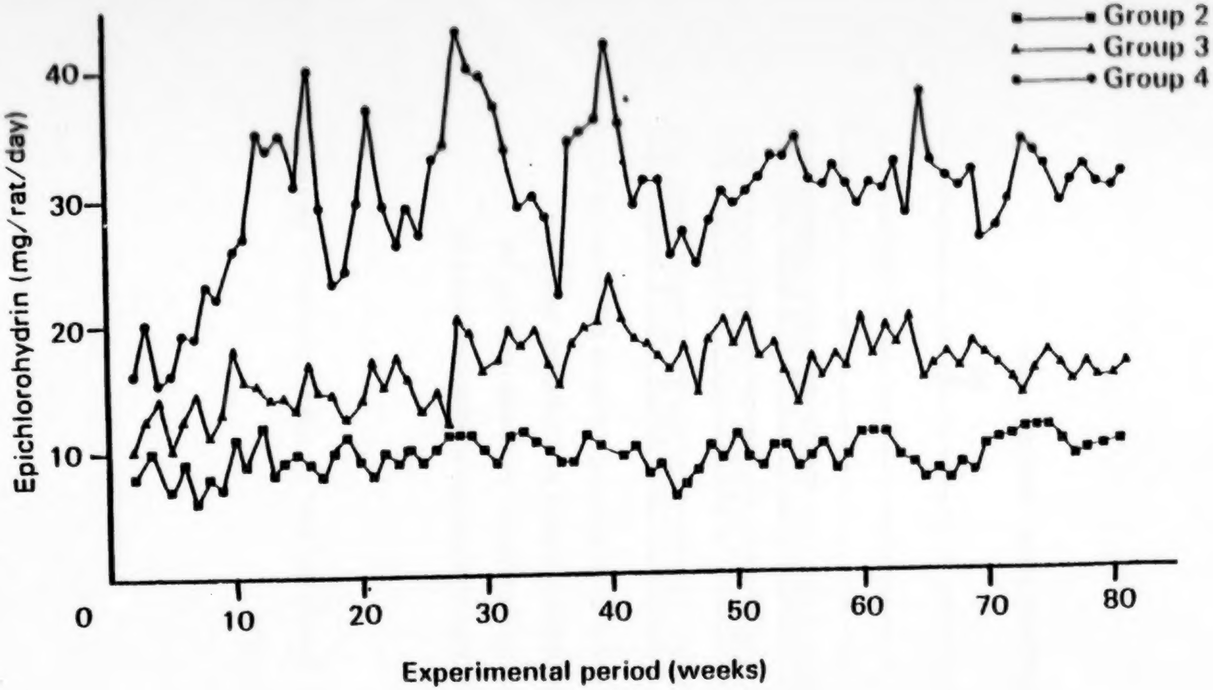


Figure 7.5. Intake of epichlorohydrin in drinking water by male Wistar rats. The lines represent the 375 ppm (■), 750 ppm (▲), and 1500 ppm (●) dose groups. Epichlorohydrin intake is calculated from known initial water concentrations and water consumption. Epichlorohydrin water hydrolysis during the 24 hours between new solution preparation was not measured; therefore, intakes of epichlorohydrin have probably been overestimated by an undetermined quantity.

SOURCE: Kawabata, 1981.

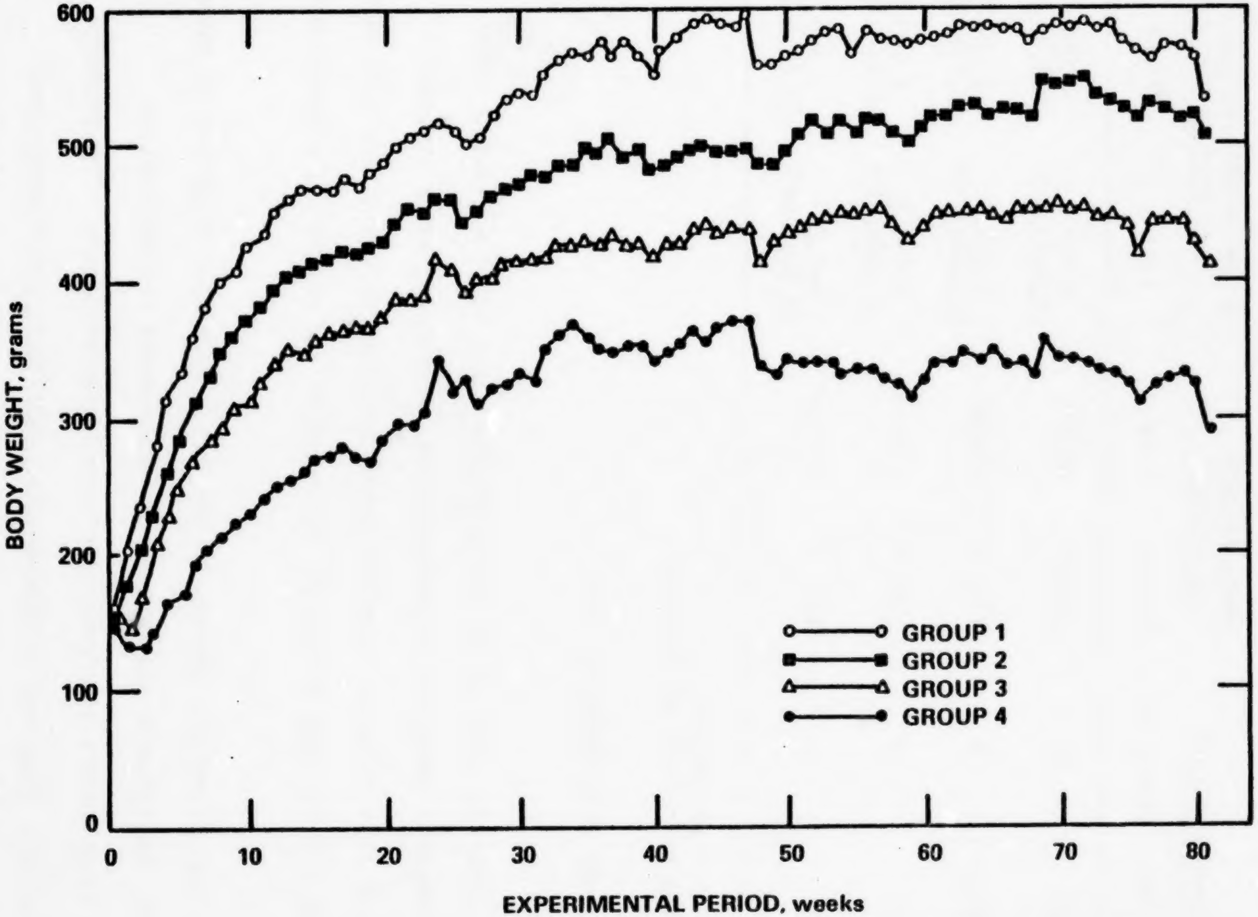


Figure 7-6. Effect of epichlorohydrin treatment of body weight in male Wistar rats. The curves represent untreated controls (○), and dose groups given 375 ppm (■), 750 ppm (△), and 1500 ppm (●).

SOURCE: Kawabata, 1981.

TABLE 7-3. KIDNEY WEIGHTS AND KIDNEY/BODY WEIGHT RATIOS IN MALE WISTAR RATS GIVEN EPICHLOROHYDRIN IN DRINKING WATER FOR 81 WEEKS

Dose (ppm)	Number of rats		Body weight[a] (g ± S.D.)		Organ weights (g ± S.D.)[a] (% of body weight)	
	Initial	Effective[b]	Initial	Final	Left	Right
0	18	10	157±10	595±75	1.7±0.2 (0.31±0.07)	1.6±0.2 (0.31±0.07)
375	18	9	159± 6	494±45	2.2±0.6[c] (0.44±0.11)[c]	2.1±0.4[c] (0.31±0.08)
750	18	10	157± 8	415±46[c]	2.1±0.2[c] (0.52±0.07)[c]	2.2±0.2[c] (0.53±0.05)[c]
1500	18	12	160± 7	295±46[c]	1.9±0.2[c] (0.66±0.09)[c]	1.9±0.2[c] (0.65±0.09)[c]

[a] S.D. = standard deviation.
[b] Based on rats sacrificed at 81 weeks.
[c] $P < 0.05$.

SOURCE: Adapted from Kawabata, 1981.

compared to controls. The relationship between these blood analysis results and epichlorohydrin treatment is presently not understood, and a stronger examination of this response to epichlorohydrin treatment could have been made if blood analyses had been done prior to treatment and periodically throughout the study.

Dose-related pathologic changes in the forestomach were diagnosed as shown in Table 7-4. Macroscopic examination of forestomachs revealed uneven protuberant large tumors and "countless" small nodular tumors. The lesions were histologicaly diagnosed as hyperplasia, papilloma, and squamous cell carcinoma. Both localized and diffuse hyperplasia were observed. Proliferation of squamous epithelium and multistage stratification of basal cells were observed

TABLE 7-4. NUMBER OF FORESTOMACH TUMORS AND INCIDENCE IN MALE WISTAR RATS GIVEN EPICHLOROHYDRIN IN DRINKING WATER FOR 81 WEEKS

Dose (ppm)	Number of rats		Number of tumors in forestomach per rat				Histological findings on squamous epithelium (%)		
				Tumor size (mm)					
	Initial	Effective[a]	Total	>5	2-5	<2	Hyperplasia	Papilloma	Carcinoma
0	18	10	0	0	0	0	0 (0)	0 (0)	0 (0)
375	18	9	5.6 ± 8.4	0	0.1 ± 0.3	5.4 ± 8.2	7 (77.8)	0 (0)	0 (0)
750	18	10	9.9 ± 12.8	0.4 ± 0.5	1.2 ± 1.2	8.3 ± 12.0	9 (90.0)	1 (10.0)	1 (10.0)
1500	18	12	32.8 ± 24.0	0.8 ± 1.0	4.4 ± 3.6	27.6 ± 21.3	12 (100.0)	7 (58.3)[b]	2 (16.7)

[a] Effective groups include terminally sacrificed animals only.

[b] $p = 0.005$ versus controls. For papillomas and carcinomas combined, $P < 0.001$ by one-tailed Fisher's Exact Test using terminal sacrifice only.

SOURCE: Konishi et al., 1980.

in hyperplastic regions. Papillomas consisted of squamous epithelium projecting into the lumen. Marked keratinization with little nuclear division was apparent in the papillomas. Carcinomas were characterized as highly differentiated, keratinized squamous epithelium that proliferated and invaded the basal membrane. Irregularly sized nuclei and characteristics of nuclear division were common in the carcinomas. Metastases of the carcinomas were not found. Additional tumor findings included squamous cell carcinomas of the oral cavity in two rats given 1,500 ppm epichlorohydrin, and interstitial cell tumors of the testes in two to four rats in each group, with no relation to dose.

The results of the study by Konishi et al. (1980) and Kawabata (1981) provide contributing evidence for carcinogenic activity of epichlorohydrin by the oral route of administration in the forestomach of male Wistar rats. However, there were several problems with the study. First, a stronger indication of carcinogenicity could possibly have been obtained if the study protocol had included larger numbers of animals and lifetime treatment and observation. Second, it is not known whether pathologic changes suggestive of carcinogenic activity of epichlorohydrin were also evident in animals that died during the study, since pathologic data for these animals were considered unreliable by the authors. Third, the dose levels in water of epichlorohydrin used were toxic to the rats, as indicated by the reduction in body weight and the need to periodically stop treatment after 60 weeks; this indicates that the MTD was exceeded. Fourth, the epichlorohydrin had a half-life in water of 1,000 minutes (0.69 day) in this experiment, and yet solutions were made daily; epichlorohydrin dosage was thus likely to have been overestimated.

Nonetheless, induction of forestomach neoplasia as seen in this Japanese study by a direct action of epichlorohydrin is supported by: 1) a Dutch oral gavage study (forestomach tumors), 2) other studies discussed herein that indicate a direct tumorigenic action of epichlorohydrin at other exposure sites, and 3) the chemical nature of epichlorohydrin as an alkylating agent.

The upper-bound unit risk can be estimated on the basis of the Konishi et al. study, but such an estimate will be of limited reliability because of the above-described problems with the study.

7.1.2.3 Oral Administration-Gavage: Rat--G.J. van Esch (Rijsinstitut voor de Volksgezonheid Bilthoven, 1982) has written a laboratory report on the induction of forestomach lesions in SPF-derived Wistar RIV:Tox rats as a result of epichlorohydrin exposure by gavage at rates of 2 and 10 mg/kg body weight/day (administered freshly made in water). Controls and both treated groups all had 50 males and 50 females. As of October 1984, this preliminary report had not been completed, peer reviewed, or published.

Intergroup comparisons showed no compound-related effects on weight during the course of the 104-week experiment. Females showed increased early mortality (up to 40 weeks) (Table 7-5) as compared to males. Increased mortality in both males and females in the 10-40 week period was attributed by van Esch to "hairballs" or trichobezoars in the stomach (as previously seen in other experiments). This situation was corrected by diet change with different fiber content at 56 weeks, after which the mortality rate was normal. The survival to 104 weeks (2 years) is judged to be normal for this strain or rat.

The hematology findings were unremarkable except in the case of 10-mg/kg dosed females, which showed decreased leukocyte counts (mainly eosinophilic cells). The male leukocyte counts seemed to be somewhat decreased, but the variation in the white cell counts was too great in males for this determination to be made with certainty.

After 20 months of exposure, all but one rat in the high-dose group (10 mg/kg) showed macroscopically neoplastic lesions in the forestomach mucosa, which varied from small protrusions to rough surfaces to verrucous masses

TABLE 7-5. CUMULATIVE MORTALITY OF RATS GIVEN EPICHLORОHYDRIN BY GAVAGE

	Periods of 10 weeks											Animals killed in following 32 weeks	
Groups	0-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90	91-100	100-104		
	Numbers of animals[a]												
Females													% Survival to 2 years
Controls	0	0	2 (2)[b]	10	10	10	10	10	12	17	19	31	62
2 mg ECH/kg bw.	1	3 (2)	6 (4)	18 (11)	19	21	21	23	24	27	30	20	40
10 mg ECH/kg bw.	1	3 (3)	9 (5)	18 (9)	19	22	24	25	26	28	28	22	44
Males													
Controls	0	0	0	1	1	1	1	1	1	9	11	39	78
2 mg ECH/kg bw.	0	1	1	2	3	3	4	6	8	18	19	31	62
10 mg ECH/kg bw.	0	0	0	5 (1)[c]	5	6	6	6	8	19	21	29	58

[a] Fifty animals initially in all groups.
[b] Numbers in parentheses indicate the numbers of animals with hairballs in the intestines.
[c] Hairballs in the stomach.

SOURCE: van Esch, 1982.

occupying extensive luminal space to masses with ulcerations and necrosis. In several cases, the stomach wall was observed to be thickened. The low-dose group (2 mg/kg) showed macroscopically far fewer neoplastic lesions of the forestomach, and the lesions were less extensive in pattern, with the first lesion seen after 22 months. Only one control rat showed a macroscopic neoplastic lesion. The observed incidences of forestomach macroscopic changes at autopsy are given in Table 7-6.

TABLE 7-6. INCIDENCE OF RAT FORESTOMACH MACROSCOPIC NEOPLASTIC LESIONS

Sex	0 mg/kg	2 mg/kg	10 mg/kg
Male	0/50	6/50	39/50
Female	1/50	1/50	23/49

SOURCE: van Esch, 1982.

Histopathology showed forestomach proliferative changes mainly in the 10-mg/kg group (HDT). Table 7-7 shows the chronology of discovery of forestomach hyperplasia, papillomas, and carcinomas. The summed histopathologic occurrence of carcinogenic activity (papillomas and carcinomas) by epichlorohydrin in the rat forestomach in the van Esch study is shown in Table 7-8.

Distal tumors were not described by van Esch, but the lung was described as being tumor-free (no aspiration-induced tumors or metastases to the lung). One *in situ* tumor of the esophagus was mentioned in the high-dose group.

This study presents better oral exposure incidence data in the rat forestomach than the Konishi et al. study because more rats per group were used, the MTD was apparently not exceeded as in the Konishi et al. study, and health

TABLE 7-7. HISTOPATHOLOGY OF PROLIFERATIVE LESIONS IN THE FORESTOMACH OF RATS GIVEN EPICHLOROHYDRIN (ECH) BY GAVAGE

Period (months)	0-12	12-14	14-16	16-18	18-20	20-22	22-24	24-26	26-28	28-30	cumulative
Group I control females											
no. of animals died or killed[a]	10(1)				2	3	4	6	10(1)	15(1)	50(3)
Hyperplasia									2	1	3
Papilloma						1	1				2
Carcinoma											0
Group I control males											
no. of animals died or killed[a]	1				1	4	4	4	15	21	50
Hyperplasia								1	1	3	5
Papilloma					1						1
Carcinoma											0
Group II, 2 mg ECH/kg BW, females											
no. of animals died or killed[a]	19(5)	2		1	1	1	5	6(1)	3	12	50(6)
Hyperplasia	1						1	3	2	5	12
Papilloma	1									1	2
Carcinoma						1				1	2
Group III, 2 mg ECH/kg BW, males											
no. of animals died or killed[a]	3		1	2	2	9(1)	2	7	12	12	50(1)
Hyperplasia	2		1	1		3	1	2	7	7	24
Papilloma							1		2	3	6
Carcinoma								3	1	2	6
Group II, 10 mg ECH/kg BW, females											
no. of animals died or killed[a]	19(6)	2(1)	2(2)	2(1)			2	8	3	11	49(10)
Hyperplasia	5	1		1							7
Papilloma											0
Carcinoma							2	8	3	11	24
Group III, 10 mg EHC/kg BW, males											
no. of animals died of killed[a]	5(1)	1		1	2	6(1)	6(1)	8	13	8	50(3)
Hyperplasia	1	1			1	2		1			6
Papilloma				1			1				2
Carcinoma						3	4	7	13	8	35

In parentheses: number of animals from which tissue was not available for histopathological examination, e.g., due to advanced autolysis.

[a] Killed in moribund state or at termination of the experiment.

SOURCE: van Esch, 1982.

TABLE 7-8. HISTOPATHOLOGY OF PAPILLOMAS AND CARCINOMAS IN THE FORESTOMACH OF RATS GIVEN EPICHLOROHYDRIN BY GAVAGE[a]

Sex	0 mg/kg	2 mg/kg	10 mg/kg
Male	1/49 (2.0%)	12/45 (26.7%)[b]	37/42 (88.1%)[c]
Female	2/38 (5.3%)	4/28 (14.3%)	24/26 (92.3%)[c]

[a] All animals that died prior to appearance of first tumor and those for which no histologic examination was performed have not been included.
[b] $P < 0.01$
[c] $P < 0.00001$

SOURCE: van Esch, 1982.

status and survival were better. Even though the early high mortality from trichobezoar obstruction could indicate a diet-treatment interaction effect, the Carcinogen Assessment Group (CAG) concludes that the van Esch epichlorohydrin-induced response in the rat forestomach is strong qualitative evidence (test for linear trend, $P < 0.0001$ for both sexes) that epichlorohydrin induces carcinogenic activity at 2 and 10 mg/kg body weight by the oral route of administration.

7.1.2.4 Dermal Exposure: Mouse--Van Duuren et al. (1974) reported the results of a topical application study of epichlorohydrin on female ICR/Ha Swiss mice (6-8 weeks of age). The epichlorohydrin sample (Eastman Organic Chemicals) was purified by distillation and checked for purity by infrared spectroscopy, nuclear magnetic resonance spectroscopy, and gas chromatography; the epichlorohydrin sample was 99.8 percent pure (personal communication with B.L. Van Duuren). Dose selection was based on the results of preliminary 4-week tests, and the highest possible doses producing minimal cytotoxicity were used.

Fifty mice received 2 mg epichlorohydrin in 0.1 mL acetone thrice weekly on the clipped dorsal skin. Thus, epichlorohydrin was tested as a complete carcinogen. The study lasted for 580 days, and the median survival time was 506 days. No skin tumors were observed, and it is concluded that epichlorohydrin is not a complete carcinogen.

Weil et al. (1963) painted one "brushful" of undiluted epichlorohydrin (purity not reported) onto the clipped dorsal skin of 40 C3H strain mice, initially 90 days old, thrice weekly for life. Thirty were alive at 17 months, and one survived for 25 months. No local or distant tumors due to the effect of epichlorohydrin were found in this study, which corresponds to the results of the repeated topical application study of epichlorohydrin in female ICR/Ha mice by Van Duuren et al. (1972a, b; 1974) described previously. The mice used by Weil et al. (1963) were initially 90 days old, which did not allow an evaluation of carcinogenicity during early growth of the animals. A "brushful" does not give any indication of the actual dose applied. This is supportive evidence that epichlorohydrin is not a complete carcinogen.

7.1.2.5 Initiation-Promotion: Mouse--In an initiation-promotion study on mouse skin, Van Duuren and coworkers (1974) applied single doses of 2 mg epichlorohydrin (99.8 percent pure) in 0.1 mL acetone to the dorsal skin of 30 female ICR/Ha mice, followed 2 weeks later by thrice-weekly skin applications of 2.5 ug phorbol myristate acetate in 0.1 mL acetone for the duration of the experiment (median survial > 385 days). Nine of thirty (9/30) mice developed skin papillomas (the first observed at 92 days), and one mouse developed a skin carcinoma. Of 30 control mice treated with phorbol myristate acetate alone, three developed papillomas (the first at 224 days), whereas no tumors occurred in 30 solvent-treated controls. Thus, epichlorohydrin was shown to be a tumor initiator, requiring complementation by a promoter in this system. Qualitatively, it may be surmised that epichlorohydrin is an initiator and not a good promoter or not a promoter at all. In relation to the quantitative risk assessment, these data suggest that epichlorohydrin acts early in the carcinogenic sequence.

7.1.2.6 Subcutaneous or Intraperitoneal Administration: Mouse--For an assay by subcutaneous injection into the flank, 50 mice were given 1 mg epichlorohydrin in 0.5 mL tricaprylin (highest possible dose producing minimal cytotoxicity in a 4-week preliminary test) once each week for 580 days. Median survival time was 486 days. Van Duuren and coworkers (1974) reported that six mice developed local sarcomas and one had a local adenocarcinoma ($P \leq 0.05$), whereas only one local sarcoma occurred in 50 tricaprylin-treated controls.

In an intraperitoneal assay by Van Duuren et al. (1974), 30 mice received weekly injections into the lower abdomen of 1 mg epichlorohydrin in 0.05 mL of tricaprylin for 450 days. None of the mice developed local sarcomas, but 11 had papillary lung tumors. Of 30 tricaprylin-treated control mice, 10 had papillary lung tumors and one had a local sarcoma. Thus, epichlorohydrin produced local sarcomas at the site of subcutaneous injection but did not produce distant tumors after intraperitoneal injections.

Kotin and Falk (1963) administered single subcutaneous injections of 5 uM (462 ug) of epichlorohydrin in 0.1 mL ethyl laurate or tricaprylin to 30 C3H-strain mice, which were observed along with solvent-treated control mice for 2 years. Of the experimental mice, four showed malignant lymphomas within 6 months, one showed a skin papilloma after 11.5 months, one showed a hepatoma after 13 months, and one showed two lung adenomas after 24 months. However, survival was poor (12 mice died during the first year) and, except for the papilloma, the tumors were of similar types and not significantly higher in frequency than those in the control group. Animals were given only one treatment with a rather low dose of epichlorohydrin at the beginning of the study; this dosing procedure appears weak for carcinogenicity testing compared to a stronger challenge of repeated treatment over a lifetime at doses as high as those maximally tolerated.

These studies indicated that epichlorohydrin is an administration-site or proximal-site animal carcinogen, but that it is not transported to form distal tumors. The tumors that are formed by epichlorohydrin apparently do not metastasize.

7.1.3 Epidemiologic Studies

A retrospective cohort mortality study of epichlorohydrin workers was conducted for Shell Oil Company by Dr. Phillip Enterline of the University of Pittsburgh (Enterline, 1978, 1981). The cohort of 864 comprised workers from Shell plants at Norco, Louisiana, and Deer Park, Texas. Deaths were compared by cause with those expected in Louisiana and Texas, respectively. Results were analyzed by vital status as of December 31, 1977 (reported by Enterline in 1978) and in the most recent update by vital status as of December 31, 1979 (reported by Enterline in 1981) for the cohort exposed to epichlorohydrin for at least one quarter before January 1, 1966. The Carcinogen Assessment Group (CAG) previously reported on the 1977 update (CAG, 1980). Those data (see Table 7-9 plus footnote) showed less observed mortality than expected (54 versus 97.3, respectively) but also showed an increase (which was not statistically significant) in both respiratory cancer and leukemias with overall standardized mortality ratios (SMRs) of 146.2 and 224.7, respectively. Furthermore, the data published in 1978 showed an apparent increase with increasing latent period since, of the 12 respiratory cancer or leukemia deaths, 11 occurred in workers 15 years or more after first exposure (Table 7-9). Even though these increases were not statistically significant, the trend provided reason for concern that increasing observation time would produce more of these cancers, leading to positive conclusions about the carcinogenicity of epichlorohydrin in humans.

TABLE 7-9. COMPARISON OF MORTALITY IN ENTERLINE'S EPICHLOROHYDRIN STUDY UPDATES BY CAUSE AND BY LATENCY (1978 versus 1981)[a]

		All cases		Respiratory cancer		Leukemia	
	Time since first exposure	Observed/ Expected	SMR	Observed/ Expected	SMR	Observed/ Expected	SMR
Enterline							
(1978)	Overall	54/97.3	55.5	10/6.8	146.2	2/0.9	224.7
	< 15 years	19/45.8	41.5	1/2.2	45.9	0/0.5	0
	≥ 15 years	35/51.6	67.9	9/4.7	193.1	2/0.4	500
(1981)	Overall	65/115.7	56.2	10/8.7	114.1	2/1.0	194.2
	< 15 years	19/46.0	41.3	1/2.2	45.0	0/0.5	0
	≥ 15 years	46/69.8	65.9	9/6.5	137.8	2/0.5	377.4

[a] Included in the 1981 report are two additional deaths missed in the 1978 reports--one due to lung cancer and one due to heart disease. The numbers above referring to the 1978 report include this correction.

However, the most recent data (Enterline, 1981) have produced a reversal of the trend of respiratory cancers and leukemia deaths. This is shown in Tables 7-9 and 7-10. In this latest 2-year followup period, 1978-1979, there were 11 additional deaths, only one of which was due to cancer, and this was not respiratory cancer or leukemia. As can be seen with respect to the SMRs for both respiratory cancers and leukemia, this most recent update has shown decreases in both of the overall SMRs, and especially in those for the group with greater than 15 years since first exposure. However, neither of these SMRs is statistically significant.

In the most recent update, Enterline (1981) also presented a smoking history of 12 of the cancer deaths. He found that for the 10 deaths diagnosed as lung cancer deaths on death certificates, 7 individuals were known smokers, 1 was a nonsmoker, and 2 had unknown smoking histories. This confounding factor makes it even more difficult to assert a positive causal relationship between epichlorohydrin and human lung cancer.

Additionally, Enterline considered the severity of epichlorohydrin exposure. Regarding the group with at least 15 years since first exposure, he stratified "heavy to moderate" versus "light to nil" groups. This analysis failed to show a dose-response trend, as the death ratios for cancer in both groups were similar.

Finally, there is a problem of exposure to multiple chemicals. This is examined in two separate studies by Enterline that share some of the same cohort (Table 7-11). In the 1981 study, Enterline provided a further analysis contrasting the mortality experience of 124 men from Deer Park who had prior exposure in the isopropyl alcohol (IPA) unit.* The results show that the

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*The exposures to IPA and epichlorohydrin were considered by the CAG in its most recent report (1980). It was concluded that the confounding effects between the exposures detracted from the significance of the findings.

TABLE 7-10. OBSERVED AND EXPECTED DEATHS AND SMRs AMONG 863 MALES EXPOSED FOR MORE THAN THREE MONTHS IN THE MANUFACTURE OF EPICHLOROHYDRIN, BY TIME SINCE FIRST EXPOSURE NORCO, LOUSIANA AND DEER PARK, TEXAS 1948-1979[a]

Causes of death	Total			Time since first exposure					
				≥ 15 years			< 15 years		
	OBS	EXP	SMR	OBS	EXP	SMR	OBS	EXP	SMR
All causes	65	115.72	56.2	46	69.75	65.9	19	45.97	41.3
All cancers (140-205)	16	21.73	73.6	15	14.94	100.4	1	6.79	14.7
Respiratory cancers (160-164)	10	8.74	114.4	9	6.53	137.8	1	2.22	45.0
Leukemias (204)	2	1.03	194.2	2	0.53	337.4	0	0.49	0
All other cancers	2	11.96	33.4	4	7.88	50.7	0	4	0
All other causes	49	93.99	52.1	31	54.81	56.6	18	39.18	45.9
Total no. of men		863			824			863	
Man-years		19,909.9			7,406.8			12,503.1	

[a] A reexamination of both the cohort and the death certificates by Enterline has led to slight changes in the numbers of the previous update. Specifically, even though only nine lung cancer deaths were presented in the previous report, a reexamination showed that actually ten had occurred. None occurred during the update 1978-1979.

SOURCE: Enterline, 1981.

TABLE 7-11. COMPARISON OF MORTALITY IN EPICHLOROHYDRIN (ECH) ALONE, AND COMBINED WITH ISOPROPYL ALCOHOL (IPA) EXPOSURE GROUPS IN DEER PARK, TEXAS

Exposure group	Number	All cases Observed/ Expected	SMR	All cancer Observed/ Expected	SMR	Respiratory cancer Observed/ Expected	SMR
Enterline							
(1981)							
ECH (alone)	308	38/60.89	62.4	11/11.79	93.3	3/4.61	65.1
IPA and ECH[a]	124	16/23.24	68.9	5/4.68	107.0	4/1.86	214.8
(1980)							
IPA (alone)	350	24/44.30	54.2	5/8.21	60.9	2/3.16	63.3
IPA and ECH[a]	125	16/22.64	70.7	5/4.37	114.4	4/1.71	233.9

[a] These represent the same cohort (except for one unidentified man) with 1 year's additional values.

SOURCE: Enterline, 1980 and 1981.

respiratory cancer SMR is much higher in the group exposed in the IPA unit (to chemicals other than epichlorohydrin) than in the group exposed to epichlorohydrin alone (SMRs = 214.8 versus 63.3, respectively).

While the above data suggest that the IPA process is responsible for the respiratory cancer increase, an additional study by Enterline of the IPA cohort suggests a different interpretation. This is the 1980 report on the mortality experience of a cohort of 433 men who worked in the IPA unit at Deer Park, Texas, from its startup in 1941 to 1965 (Enterline, 1980). Table 7-11 shows the mortality patterns for all causes, all cancers, and all respiratory cancers. As can be seen, the IPA plus epichlorohydrin combined group had higher SMRs than the IPA group alone in all three categories, with the major increase being in respiratory cancer deaths. Also, the epichlorohydrin group alone had approximately the same respiratory cancer mortality as the IPA group alone.

The conclusion made by the CAG is that the most recent update of the Enterline data has provided less clear evidence for the human carcinogenicity of epichlorohydrin. The evidence for the carcinogenicity of epichlorohydrin includes increased respiratory cancer with increasing latent period and the higher respiratory SMR in cancer in the combined IPA plus epichlorohydrin group versus the IPA group alone. Further, there was elevated respiratory cancer in both epichlorohydrin production plants (CAG, 1980). Contrary to this evidence, there are no statistically significant increases and actually a decreased SMR in the latest 2-year update as compared to the earlier update. Also, the increase in respiratory cancer SMR in the combined IPA plus epichlorohydrin exposure group, as compared with either the epichlorohydrin group alone or the IPA group alone, suggests that the interaction between IPA and epichlorohydrin exposure leads to increased respiratory cancer. Significantly, in the Deer Park, Texas epichlorohydrin alone subgroup of 350, there was no increase in respiratory cancer versus controls (see also Enterline, 1982). Considering, in addition, the confounding factor of smoking, the CAG is of the opinion that these studies provide only limited evidence for the human carcinogenicity of epichlorohydrin.

Shellenberger et al. (1979) conducted a restrospective cohort mortality study of 533 white male full-time Dow Chemical Company employees who had potential epichlorohydrin exposure in a production area for at least 1 month between October 1957 (the date on which commercial production of epichlorohydrin began in the Dow Chemical Company, Texas Division) and November 1976. In all, there were 12 deaths during this period: one cancer death from adenocarcinoma of the stomach, one death due to metastatic malignant melanoma, five deaths due to cardiovascular diseases, and five deaths from accidents. The two observed cancer deaths were less than the number expected (3.50) for the entire group. In a further breakdown, Shellenberger et al. subdivided the cohort, enumerating the 202 persons with at least 1 year of epichlorohydrin exposure and holding at least one job in which epichlorohydrin exposure was estimated to be >1 ppm. Neither of the two cancers was from this group.

Although this study was negative with respect to cancer mortality, it has drawbacks relative to carcinogenicity assessment. First, only 2 percent (12/553) of the cohort died during the 11-year follow-up period. This would have been only 1.3 percent had it not been for the five accidental deaths. The expected death rate for accidents was larger than that for cancer, indicating a very young cohort. The actual average age at the end of the follow-up period was only 39 years, and 61.8 percent of the cohort was less than 40 years of age as of the cutoff date. The average duration of exposure for the study cohort was only 3 years, with 43.9 percent exposed less than 1 year and 58.7 percent exposed less than 2 years. The average interval since first exposure was only 7.7 years, with 47.7 percent having less than 7 years from first exposure to the end of the study. This epidemiologic study on epichlorohydrin, while negative, is inadequate for the evaluation of carcinogenicity because of its low exposures, short exposure durations, short latent period, and very young age of the cohort.

Tassignon et al. (1983) presented a report to the EPA detailing an historic prospective study of workers exposed to epichlorohydrin at four European plants that manufactured epichlorohydrin and epichlorohydrin-derived chemicals. A total of 606 males, who had had at least 1 year of exposure to epichlorohydrin at least 10 years prior to the final date of the study on December 1978, were analyzed for their mortality experience. The combined results showed 10 observed deaths versus 18.4 expected (SMR = 54.3), and four cancer deaths versus five expected (SMR = 80). While these four cancers were all from different sites and provide no evidence of an association between epichlorohydrin and cancer, the study by itself lacks the power to establish the safety of epichlorohydrin. Several limiting factors are: 1) the relatively small study size, especially the size of the group (274) with at least 10 years of exposure; 2) the low average age of the cohort (42 years); 3) the lack of any analysis by time since first exposure (the authors state that they would have preferred a longer minimal observation period but that the cohort would then have become very small); and 4) personal exposure measurements taken in 1977 and 1978 showing that exposure levels were low (at or below 1 ppm, 8-hour time weighted average, even though earlier exposures are stated by the authors to have been considerably higher.

Finally, there is the peculiar result that the cohort yielded zero dead and 90 live retirees. Considering that retirees generally have about 5 percent mortality per year, this is a highly improbable result. However, the paper does not report either the person-years of these retirees, their length of exposure, or their average age, with the result that further analysis is impossible. Furthermore, if the average retiree's age is 65, then the average age of the other 516 men in the cohort would have to be low--around 38--in order for the average age of the total cohort to be 42 years. A more complete report would perhaps answer these questions. In short, this study appears to suffer from many of the same deficiencies as the Shellenberger et al. study.

7.1.4 Quantitative Estimation

This section deals with the incremental unit risk for epichlorohydrin in air and water, and the potency of epichlorohydrin relative to other carcinogens that the CAG has evaluated. The incremental unit risk estimate for an air or water pollutant is defined as the incremental lifetime cancer risk to an individual due to continuous exposure from birth throughout life to a concentration of 1 ug/m^3 of the agent in the air breathed, or to 1 ug/L in drinking water. This calculation estimates in quantitative terms the impact of the agent as a carcinogen. Unit risk estimates are used for two purposes: 1) to compare the carcinogenic potency of several agents with each other, and 2) to give a crude indication of the population risk that might be associated with air or water exposure to these agents if the actual exposures were known.

7.1.4.1 Procedures for Determination of Unit Risk--The data used for the quantitative estimate are taken from one or both of the following: 1) lifetime animal studies, and 2) human studies where excess cancer risk has been associated with exposure to the agent. In animal studies it is assumed, unless evidence exists to the contrary, that if a carcinogenic response occurs at the dose levels used in the study, then responses will also occur at all lower doses, with an incidence determined by an extrapolation model.

There is no solid scientific basis for any mathematical extrapolation model that relates carcinogen exposure to cancer risks at the extremely low concentrations that must be dealt with in evaluating environmental hazards. For practical reasons, such low levels of risk cannot be measured directly either by animal experiments or by epidemiologic studies. We must, therefore, depend on our current understanding of the mechanisms of carcinogenesis for guidance as to which risk model to use. At the present time the dominant view of the carcinogenic process involves the concept that most agents that cause cancer also cause irreversible damage to DNA. This position is reflected by the fact that a very large proportion of agents that cause cancer are also mutagenic. There is reason to expect that the quantal type of biological response, which is characteristic of mutagenesis, is associated with a linear non-threshold dose-response relationship. Indeed, there is substantial evidence from mutagenicity studies with both ionizing radiation and a wide variety of chemicals that this type of dose-response model is the appropriate one to use. This is particularly true at the lower end of the dose-response curve; at higher doses, there can be an upward curvature, probably reflecting the effects of multistage processes on the mutagenic response. The linear nonthreshold dose-response relationship is also consistent with the relatively few epidemiologic studies of cancer responses to specific agents that contain enough information to make the evaluation possible (e.g., radiation-induced leukemia, breast and thyroid cancer, skin cancer induced by arsenic in drinking water, liver cancer induced by aflatoxin in the diet). There is also some evidence from animal experiments that is consistent with the linear nonthreshold model (e.g., liver tumors induced in mice by 2-acetylaminofluorene in the large-scale ED_{01} study at the National Center for Toxicological Research, and the initiation stage of the two-stage carcinogenesis model in rat liver and mouse skin).

Because its scientific basis, although limited, is the best of any of the current mathematical extrapolation models, the linear nonthreshold model has been adopted as the primary basis for risk extrapolation to low levels of the dose-response relationship. The risk estimates made with this model should be regarded as conservative, representing the most plausible upper limit for the risk, i.e., the true risk is not likely to be higher than the estimate, but it could be lower.

The mathematical formulation chosen to describe the linear nonthreshold dose-response relationship at low doses is the linearized multistage model. This model employs enough arbitrary constants to fit almost any monotonically increasing dose-response data, and it incorporates a procedure for estimating the largest possible linear slope (in the 95 percent confidence limit sense) at low extrapolated doses that is consistent with the data at all dose levels of the experiment.

7.1.4.1.1 Description of the Low-Dose Extrapolation Model. Let P(d) represent the lifetime risk (probability) of cancer at dose d. The multistage model has the form

$$P(d) = 1 - \exp\left[-(q_0 + q_1 d + q_1 d^2 + \ldots + q_k d^k)\right]$$

where

$$q_i \geq 0,\ i = 0, 1, 2, \ldots, k$$

Equivalently,

$$P_t(d) = 1 - \exp\left[-(q_1 d + q_2 d^2 + \ldots + q_k d^k)\right]$$

where

$$P_t(d) = \frac{P(d) - P(0)}{1 - P(0)}$$

is the extra risk over background rate at dose d.

The point estimate of the coefficients q_i, $i = 0, 1, 2, \ldots, k$, and consequently, the extra risk function, $P_t(d)$, at any given dose d, is calculated by maximizing the likelihood function of the data.

The point estimate and the 95 percent upper confidence limit of the extra risk, $P_t(d)$, are calculated by using the computer program GLOBAL79, developed by Crump and Watson (1979). At low doses, upper 95 percent confidence limits on the extra risk and lower 95 percent confidence limits on the dose producing a given risk are determined from a 95 percent upper confidence limit, q_1^*, on parameter q_1. Whenever $q_1 > 0$, at low doses the extra risk, $P_t(d)$, has approximately the form $P_t(d) = q_1 \times d$. Therefore, $q_1^* \times d$ is a 95 percent upper confidence limit on the extra risk, and R/q_1^* is a 95 percent lower confidence limit on the dose, producing an extra risk of R. Let L_0 be the maximum value of the log-likelihood function. The upper-limit, q_1^*, is calculated by increassing q_1 to a value q_1^* such that when the log-likelihood is remaximized subject to this fixed value q_1^* for the linear coefficient, the resulting maximum value of the log-likelihood L_1 satisfies the equation

$$2\ (L_0 - L_1) = 2.70554$$

where 2.70554 is the cumulative 90 percent point of the chi-square distribution with one degree of freedom, which corresponds to a 95 percent upper limit (one-sided). This approach of computing the upper confidence limit for the extra risk $P_t(d)$ is an improvement on the Crump et al. (1977) model. The upper confidence limit for the extra risk calculated at low doses is always linear. This is conceptually consistent with the linear nonthreshold concept discussed earlier. The slope, q_1^*, is taken as an upper bound of the potency of the chemical in inducing cancer at low doses.

In fitting the dose-response model, the number of terms in the polynomial is chosen equal to (h-1), where h is the number of dose groups in the experiment, including the control group.

Whenever the multistage model does not fit the data sufficiently well, data at the highest dose is deleted and the model is refit to the rest of the data. This is continued until an acceptable fit to the data is obtained. To determine whether or not a fit is acceptable, the chi-square statistic

$$X^2 = \sum_{i=1}^{h} \frac{(X_i - N_iP_i)^2}{N_iP_i\ (1-P_i)}$$

is calculated where N_i is the number of animals in the i^{th} dose group, X_i is the number of animals in the i^{th} dose group with a tumor response, P_i is the probability of a response in the i^{th} dose group estimated by fitting the multistage model to the data, and h is the number of remaining groups. The fit is determined to be unacceptable whenever X^2 is larger than the cumulative 99 percent point of the chi-square distribution with f degrees of freedom, where f equals the number of dose groups minus the number of non-zero multistage coefficients.

For cases of partial lifetime exposure where time-to-tumor or time-to-tumor death is known, Crump and Howe (1984) have developed the multistage model to include a time-dependent dose pattern. The form of this model is one which is linear in dose and in which time has a power and form determined by both the number of assumed stages and the stage affected by the carcinogen. This model is used for the epichlorohydrin inhalation study of Laskin et al. (1980). A best fit will be determined by the method of maximum likelihood in the ADOLL1-83 computer (Crump and Howe, 1983).

7.1.4.1.2 Selection of Data. For some chemicals, several studies in different animal species, strains, and sexes, each run at several doses and different routes of exposure, are available. A choice must be made as to which of the data sets from several studies to use in the model. It may also be appropriate to correct for metabolism differences between species and for absorption factors via different routes of administration. The procedures used in evaluating these data are consistent with the approach of making a maximum-likely risk estimate. They are listed below:

1. The tumor incidence data are separated according to organ sites or tumor types. The set of data (i.e., dose and tumor incidence) used in the model is the set in which the incidence is statistically significantly higher than the control for at least one test dose level, or in which the tumor incidence rate shows a statistically significant trend with respect to dose level. The data set that gives the highest estimate of the lifetime carcinogenic risk, q_1^*, is selected in most cases. However, efforts are made to exclude data sets that produce spuriously high risk estimates because of a small number of animals. That is, if two sets of data show a similar dose-response relationship, and one has a very small sample size, the set of data having the larger sample size is selected for calculating the carcinogenic potency.

2. If there are two or more data sets of comparable size that are

identical with respect to species, strain, sex, and tumor sites, the geometric mean of q_1^*, estimated from each of these data sets, is used for risk assessment.

3. If two or more significant tumor sites are observed in the same study, and if the data are available, the number of animals with at least one of the specific tumor sites under consideration is used as incidence data in the model.

7.1.4.1.3 Calculation of Human Equivalent Dosages from Animal Data. Following the suggestion of Mantel and Schneiderman (1975), it is assumed that mg/surface area/day is an equivalent dose between species. Since, to a close approximation, surface area is proportional to the two-thirds power of weight, as would be the case for a perfect sphere, the exposure in mg/day per two-thirds power of the weight is also considered to be equivalent exposure. In an animal experiment, this equivalent dose is computed in the following manner:

Let

L_e = duration of experiment

l_e = duration of exposure

m = average dose per day in mg during administration of the agent (i.e., during l_e), and

W = average weight of the experimental animal

The lifetime exposure is then

$$d = \frac{l_e \times m}{L_e \times W^{2/3}}$$

7.1.4.1.3.1 Oral. Often exposures are not given in units of mg/day, and it becomes necessary to convert the given exposures into mg/day. For example, in most feeding studies exposure is given in terms of ppm in the diet. In these cases, the exposure in mg/day is

$$m = \text{ppm} \times F \times r$$

where ppm is parts per million of the carcinogenic agent in the diet or water, F is the weight of the food or water consumed per day in kg, and r is the absorption fraction. In the absence of any data to the contrary, r is assumed to be equal to one. For a uniform diet, the weight of the food consumed is proportional to the calories required, which in turn is proportional to the surface area, or two-thirds power of the weight, so that

$$m \propto \text{ppm} \times W^{2/3} \times r$$

or

$$\frac{m}{rW^{2/3}} \propto \text{ppm}.$$

As a result, ppm in the diet is often assumed to be an equivalent exposure between species. However, this is not justified for the present study, since the ratio of calories to food weight is very different in the diet of man as compared to laboratory animals, primarily due to differences in the moisture content of the foods eaten. It is therefore necessary to use an empirically-derived factor, f = F/W, which is the fraction of an organism's body weight that is consumed per day as food, expressed as follows:

		Fraction of body weight consumed as	
Species	W	f_{food}	f_{water}
Man	70	0.028	0.029
Rats	0.35	0.05	0.078
Mice	0.03	0.13	0.17

Thus, when exposure is given as a certain dietary or water concentration in ppm, the exposure in $mg/W^{2/3}$ is

$$\frac{m}{rW^{2/3}} = \frac{ppm \times F}{W^{2/3}} = \frac{ppm \times f \times W}{W^{2/3}} = ppm \times f \times W^{1/3}$$

7.1.4.1.3.2 Inhalation. When exposure is given in terms of mg/kg/day = $m/Wr = s$, the conversion is simply

$$\frac{m}{rW^{2/3}} = s \times W^{1/3}$$

When exposure is via inhalation, the calculation of dose can be considered for two cases where 1) the carcinogenic agent is either a completely water-soluble gas or an aerosol and is absorbed proportionally to the amount of air breathed in, and 2) where the carcinogen is a poorly water-soluble gas which reaches an equilibrium between the air breathed and the body compartments. After equilibrium is reached, the rate of absorption of these agents is expected to be proportional to the metabolic rate, which is proportional to the rate of oxygen consumption, which in turn is a function of surface area.

Case 1--Agents that are in the form of particulate matter or virtually completely absorbed gases, such as sulfur dioxide, can reasonably be expected to be absorbed proportionally to the breathing rate. In this case the exposure in mg/day may be expressed as:

$$m = I \times v \times r$$

where I = inhalation rate per day in m^3, v = mg/m^3 of the agent in air, and

r = the absorption fraction.

The inhalation rates, I, for various species can be calculated from the observations of the Federation of American Societies for Experimental Biology (FASEB, 1974) that 25-g mice breathe 34.5 liters/day and 113-g rats breathe 105 liters/day. For mice and rats of other weights, W (in kilograms), the surface area proportionality can be used to find breathing rates in m^3/day as follows:

$$\text{For mice, } I = 0.0345\ (W/0.025)^{2/3}\ m^3/\text{day}$$

$$\text{For rats, } I = 0.105\ (W/0.113)^{2/3}\ m^3/\text{day}$$

For humans, the value of 20 m^3/day is adopted as a standard breathing rate by the International Commission on Radiological Protection (ICRP, 1977)*.

The equivalent exposure in $mg/W^{2/3}$ for these agents can be derived from the air intake data in a way analogous to the food intake data. The empirical factors for the air intake per kg per day, $i = I/W$, based upon the previously stated relationships, are tabulated as follows:

Species	W	i = I/W
Man	70	0.29
Rats	0.35	0.64
Mice	0.03	1.3

Therefore, for particulates or completely absorbed gases, the equivalent exposure in $mg/W^{2/3}$ is

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*From "Recommendation of the International Commission on Radiological Protection," page 9. The average breathing rate is 10^7 cm^3 per 8-hour workday and 2×10^7 cm^3 in 24 hours.

$$d = \frac{m}{W^{2/3}} = \frac{Ivr}{W^{2/3}} = \frac{iW^{1/3}vr}{W^{2/3}} = iW^{1/3}vr$$

In the absence of experimental information or a sound theoretical argument to the contrary, the fraction absorbed, r, is assumed to be the same for all species.

Case 2--The dose in mg/day of partially soluble vapors is proportional to the O_2 consumption, which in turn is proportional to $W^{2/3}$ and is also proportional to the solubility of the gas in body fluids, which can be expressed as an absorption coefficient, r, for the gas. Therefore, expressing the O_2 consumption as $O_2 = k\ W^{2/3}$, where k is a constant independent of species, it follows that:

$$m = k\ W^{2/3} \times v \times r$$

or

$$d = \frac{m}{W^{2/3}} = kvr$$

As with Case 1, in the absence of experimental information or a sound theoretical argument to the contrary, the absorption fraction, r, is assumed to be the same for all species. Therefore, for these substances a certain concentration in ppm or ug/m^3 in experimental animals is equivalent to the same concentration in humans. This is supported by the observation that the minimum alveolar concentration necessary to produce a given "stage" of anesthesia is similar in man and animals (Dripps et al., 1977). When the animals are exposed via the oral route and human exposure is via inhalation or vice versa, the assumption is made, unless there is pharmacokinetic evidence to the contrary, that absorption is equal by either exposure route.

7.1.4.1.4 Calculation of the Unit Risk from Animal Studies. The risk associated with d mg/kg$^{2/3}$/day is obtained from GLOBAL79 and, for most cases of interest to risk assessment, can be adequately approximated by $P(d) = 1 - \exp(-q_1^* d)$. A "unit risk" in units X is simply the risk corresponding to an exposure of X = 1. To estimate this value, the number of mg/kg$^{2/3}$/day corresponding to one unit of X is determined and substituted into the above relationship. Thus, for example, if X is in units of ug/m^3 in the air, then for case 1, $d = 0.29 \times 70^{1/3} \times 10^{-3}$ mg/kg$^{2/3}$/day, and for case 2, d = 1, when ug/m^3 is the unit used to compute parameters in animal experiments.

If exposures are given in terms of ppm in air, then the conversion factor to mg/m^3 is

$$1 \text{ ppm} = 1.2 \times \frac{\text{molecular weight (gas)}}{\text{molecular weight (air)}} \text{ mg/m}^3$$

Note that an equivalent method of calculating unit risk would be to use mg/kg for the animal exposures, and then to increase the jth polynomial coefficient by an amount:

$$(W_h/W_a)^{j/3} \quad j = 1, 2, \ldots, K$$

and use mg/kg equivalents for the unit risk values.

7.1.4.1.5 Adjustments for Less Than Lifespan Duration of Experiment. If the duration of experiment, L_e, is less than the natural lifespan of the test animal L, the slope q_1^*, or more generally the exponent g(d), is increased by multiplying by a factor $(L/L_e)^3$. We assume that if the average dose d is continued, the age-specific rate of cancer will continue to increase as a constant function of the background rate. The age-specific rates for humans increase at least by the second power of the age and often by a considerably higher power, as demonstrated by Doll (1971). Thus, it is expected that the cumulative tumor rate would increase by at least the third power of age. Using this fact, it is assumed that the slope q_1^*, or more generally the exponent g(d), would also increase by at least the third power of age. As a result, if the slope q_1^* [or g(d)] is calculated at age L_e, it is expected that if the experiment had been continued for the full lifespan, L, at the given average exposure, the slope q_1^* [or g(d)] would have been increased by at least $(L/L_e)^3$.

This adjustment is conceptually consistent with the proportional hazard model proposed by Cox (1972) and the time-to-tumor model considered by Crump (1979), where the probabiity of cancer by age t and at dose d is given by

$$P(d,t) = 1 - \exp[-f(t) \times g(d)].$$

It is also consistent with the partial lifetime exposure extension of the multistage model developed by Crump and Howe (1984).

7.1.4.1.6 Interpretation of Quantitative Estimates. For several reasons, the unit risk estimate based on animal bioassays is only an approximate indication of the absolute risk in populations exposed to known carcinogen concentrations. First, there are important species differences in uptake, metabolism, and organ distribution of carcinogens, as well as species differences in target site susceptibility, immunological responses, hormone function, dietary factors, and disease. Second, the concept of equivalent doses for humans compared to animals on a mg/surface area basis is virtually without experimental verification regarding carcinogenic response. Finally, human populations are variable with respect to genetic constitution and diet, living environment, activity patterns, and other cultural factors.

The unit risk estimate can give a rough indication of the relative potency of a given agent as compared with other carcinogens. The comparative potency of different agents is more reliable when the comparison is based on studies in the same test species, strain, and sex, and by the same route of exposure, preferably inhalation.

The quantitative aspect of carcinogen risk assessment is included here because it may be of use in the regulatory decision-making process, e.g., setting regulatory priorities, evaluating the adequacy of technology-based controls, etc. However, it should be recognized that the estimation of cancer risks to humans at low levels of exposure is uncertain. Because of the limited data available from animal bioassays, especially at the high dose levels required for testing, almost nothing is known about the true shape of the dose-response curve at low environmental levels. At best, the linear extrapolation model used here provides a rough but plausible estimate of the upper limit of risk; i.e., it is not likely that the true risk would be much more than the estimated risk, but it could be considerably lower. The risk estimates presented in subsequent sections should not be regarded as accurate representations of the true cancer risk even when the exposures are accurately defined. The estimates presented may, however, be factored into regulatory decisions to the extent that the concept of upper risk limits is found to be useful.

7.1.4.1.7 Alternative Methodological Approaches. The methods used by the CAG for quantitative assessment are consistently conservative, i.e., tending toward high estimates of risk. The most important part of the methodology contributing to this conservatism is the CAG's use of the linear nonthreshold extrapolation model. There are a variety of other extrapolation models that could be used, all of which would give lower risk estimates. These alternative models, the one-hit, log-probit, and Weibull models, have not been used in the following analysis, but are included for comparison in Appendix E. Another alternative method involves basing extrapolations on animal bioassay data, using either the most sensitive responses or averages of the responses of all adequately tested bioassay animals.

Extrapolations from animals to humans can also be done on the basis of relative weights rather than surface areas. The latter approach, used here, has more basis in human pharmacological responses; however, since it is not yet clear which of the two approaches is more appropriate for carcinogens, it seems appropriate to use the method in most general use, which is also the more conservative method. In the case of epichlorohydrin drinking water studies, the use of extrapolations based on surface area rather than weight increases the unit risk estimates by a factor of 5.8.

7.1.4.1.8. Model for Estimation of Unit Risk Based on Human Data. If human epidemiologic studies and sufficiently valid exposure information are available for a compound, the CAG always makes use of these data in its analyses. If these studies show a carcinogenic effect, the data are analyzed to give an estimate of the linear dependence of cancer rates on lifetime average dose, which is equivalent to the factor B_H. If the epidemiologic data show no carcinogenic effects when positive animal evidence is available, then it is assumed that a risk does exist, but that it is smaller than could have been observed in the epidemiologic study. An upper limit to the cancer incidence is then calculated, assuming hypothetically that the true incidence is just below the level of detection in the cohort studied. Whenever possible, human data are used in preference to animal bioassay data.

Very little information exists that can be utilized to extrapolate from high-exposure occupational studies to low environmental levels. However, if a number of simplifying assumptions are made, it is possible to construct a crude dose-response model whose parameters can be estimated using vital statistics, epidemiologic studies, and estimates of worker exposures.

In human studies, the response is measured in terms of the relative risk of the exposed cohort of individuals as compared to the control group. The mathematical model employed assumes that for low exposures the lifetime probability of death from lung cancer (or any cancer), P_0, may be represented by the linear equation

$$P_0 = A + B_H x$$

where A is the lifetime probability in the absence of the agent, and x is the average lifetime exposure to environmental levels in units such as ppm. The factor B_H is the increased probability of cancer associated with each unit increase of the agent in air.

If we make the assumption that R, the relative risk of lung cancer for exposed workers in comparison to the general population, is independent of the length or age of exposure but depends only upon the average lifetime exposure, it follows that

$$R = \frac{P}{P_0} = \frac{A + B\ (x_1 + x_2)}{A + B_H\ (x_1)}$$

or

$$RP_0 = A + B_H\ (x_1 + x_2)$$

where x_1 = lifetime average daily exposure to the agent for the general population, x_2 = lifetime average daily exposure to the agent in the occupational setting, and P_0 = lifetime probability of dying of cancer with no or negligible epichlorohydrin exposure.

Substituting $P_0 = A + B_H x_1$ and rearranging gives

$$B_H = P_0 (R - 1)/x_2$$

To use this model, estimates of R and x_2 must be obtained from the epidemiologic studies. The value P_0 is derived by means of life table methodology from the age-cause-specific combined death rates for males found in the 1976 U.S. Vital Statistics tables. For lung cancer, the estimate of P_0 is 0.036. This methodology is used in the following section, which deals with unit risk estimates based on human studies.

7.1.4.2 Calculation of Quantitative Estimates--

7.1.4.2.1 Unit Risk Estimates Based on Human Studies. In making a risk estimate on the basis of data from the study of Shell Oil epichlorohydrin workers, the confounding effect of epichlorohydrin and IPA exposures cannot be ignored. Neither can the fact that the SMR increase was not statistically significant. Because this study was inconclusive and not positive, it is possible only to calculate an upper bound to the risk based on the sufficient evidence of carcinogenicity in animals.

The basis for calculating a risk estimate is the one-sided upper 95 percent confidence limit of the SMR for respiratory cancer (International Classification of Diseases 160-164). Enterline's corrected cause of death classification (Enterline, 1981, Table 7), which attributes two of the 10 lung cancer deaths to other cancers, was also used. In addition, all eight of the remaining lung cancer deaths occurred after a 15-year latency period. For lung cancer, the corrected SMR increases from 0 for < 15 years to 122.5 for ≥ 15 years (8 observed vs. 6.53 expected deaths). The associated 95 percent confidence limit for these eight observed deaths is 14.4.* The corresponding SMR is $100 \times 14.4 + 6.53 = 221$. Thus, 221 was chosen as the upper 95 percent limit.

The average age of the cohort at the time of this follow-up was 50 years. The years of exposure were not given for the whole cohort, but Enterline's report gave the duration of exposure prior to January 1, 1966, and the dates of death for the eight who died from respiratory cancer. For these eight, the average length of time from beginning of exposure to death was about 19.7 years. If the known time of non-exposure between the beginning of exposure and January 1966 is subtracted, the average duration of exposure is 13.4 years. Since six of the eight had retired or left the employment of Shell Oil prior to the time of death, the actual years of exposure are fewer than 13.4.

No exposure data are given for the Shell Oil study other than to separate the workers into two exposure groups of (1) light to nil, and (2) moderate to heavy exposure. Lung cancer deaths occurred in both groups, with the heavier exposure group having a higher SMR. An exposure level of 5 ppm was chosen as an average exposure based on the following considerations: Exposure must have been less than 20 ppm, since workers reported extreme discomfort from only one hour of exposure to 20 ppm (NIOSH, 1976a, 1978). Exposure must have been more than 1 ppm, since recent plant improvements in epichlorohydrin manufacturing facilities had reduced exposures to 1 ppm or less (NIOSH, 1978). Since half of the cohort of workers had "moderate to heavy" exposure, according to Shell Oil, it was reasoned that these workers were probably exposed to more than the current Threshold Limit Value (TLV), on the basis that a company would not label as "heavy exposure" values that were at or below the TLV. The TLV current for many years was 5 ppm, so it was chosen as a reasonable average for a cohort of workers divided approximately half and half into light and heavy exposure.

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*If the observed eight deaths were from a Poisson distribution with 14.4 expected deaths, the probability of observing eight or fewer deaths is equal to 0.05.

The exposure of these workers to epichlorohydrin averaged over a lifetime is given by

$$\text{Exposure} = 5 \text{ ppm} \times \frac{8}{24} \text{ hrs} \times \frac{240}{365} \text{ days} \times \frac{13.4}{50} \text{ yrs}$$

$$\text{Exposure} = 0.29 \text{ ppm}$$

The probability of dying from respiratory cancer from a lifetime exposure to 1 ppm epichlorohydrin/m^3 air is given by

$$B_H = \frac{P_0 \ (R-1) \ X_1}{X_2}$$

where P_0, the background lifetime probability of dying from respiratory cancer in the United States, is 0.036, R is the respiratory cancer relative risk of the workers, X_1 is exposure at 1 ppm, and X_2 is the exposure experienced by the workers. Substituting the appropriate numbers, we get

$$B_H = \frac{0.036 \times (2.21 - 1) \times 1 \text{ ppm}}{0.29 \text{ ppm}} = 0.15$$

Thus, the upper 95% limit of the SMR for lung cancer based on the observed 8 deaths and the expected 6.53 deaths yields a unit risk of 0.15. To convert ppm to ug/m^3, the formula is

$$\text{ug/m}^3 = \frac{10^{-3}}{1.2\ (\text{m.w. chemical})/(\text{m.w. air})}$$

$$\text{ug/m}^3 = \frac{10^{-3}}{1.2\ (92.5)/(28.8)}$$

$$1\ \text{ug/m}^3\ \text{epichlorohydrin} = 2.59 \times 10^{-4}\ \text{ppm}$$

Thus, the upper limit of risk of death from lung cancer as a result of breathing 1 ug/m^3 epichlorohydrin is

$$P = 2.59 \times 10^{-4} \times 0.15 = 3.9 \times 10^{-5}\ (\text{ug/m}^3)^{-1}$$

These are considered to be upper-bound risk estimates, since they are based on a linear extrapolation to low doses. The lower bound of risk approaches zero in view of the uncertainties in both the qualitative evaluation and the quantitative extrapolation process. The plausibility of the upper bound is enhanced when there is clear evidence of mutagenicity, which is the case for epichlorohydrin.

7.1.4.2.2 Unit Risk Estimates Based on Animal Studies. Two positive animal oral studies are available for a quantitative risk assessment, one gavage study and one drinking water study. The van Esch (1982) gavage study showed increased papillomas and carcinomas of the forestomach, as did the drinking water study. However, the gavage study was not used in the present quantitative estimation for several reasons: (1) the report has not yet been published; (2) the diet used for the first 56 weeks of this study caused the death of animals due to "hairballs" in the stomach. The interaction of diet and epichlorohydrin with respect to stomach lesions could not be dismissed; and (3) the gavage route is not as appropriate for animal-to-man extrapolation as the drinking water route, especially for a contact carcinogen such as epichlorohydrin.

Because of the limitations discussed previously, the bioassay of epichlorohydrin in the drinking water of male Wistar rats (Konishi et al., 1980; Kawabata, 1981) may be considered a pilot study. The results of this study nevertheless are chosen as presenting adequate evidence of carcinogenicity for calculating a unit risk by the drinking water route, mainly because it is the only drinking water study that has been done to date. The pertinent cancer data, shown in Table 7-4, present a doseresponse trend for both papillomas and carcinomas of the forestomach. Tumor response of papillomas and carcinomas combined on terminal sacrifice at 81 weeks was 0/10, 0/9, 2/10, and 9/12 for the control, 375-ppm, 750-ppm, and 1,500-ppm dose groups, respectively. This increase was statistically significant at the $P < 0.001$ level for high-dose animals vs. controls, and is considered biologically significant, especially in view of the early terminal sacrifice.

Since epichlorohydrin is a direct-acting alkylating agent, this response to the forestomach can be considered a local reaction. As such, the effect is dependent not on the dose per body weight, but on the dose per square inch of forestomach area. Unfortunately, the relative surface areas of rats and humans are not comparable. Furthermore, dose to the target organ is also dependent on comparative residence time, on which no information is as yet available. The estimates of unit risk based on this study must be viewed in the light of these uncertainties.

The method chosen by the CAG to estimate human exposure to epichlorohydrin is that of determining the equivalent concentration a human must ingest in drinking water, allowing for the difference in ratio of water ingested to body weight. In formula terms this is

$$f_{\text{water, rat}} \times \text{water conc., rat} = f_{\text{water, human}} \times \text{water conc., human}$$

The use of this formula results in doses that are equal on a dose/body weight basis, as opposed to dose/(body weight)$^{2/3}$ as discussed in the methodology section. The water-to-body-weight ratios were given earlier as $f_{\text{water}} = 0.078$ for the rat and $f_{\text{water}} = 0.029$ for the human. Thus, the rat-to-human dose ratio is 0.078/0.029 = 2.7. For the rat experiment, the drinking water concentrations for the three dose groups were 375, 750, and 1,500 ppm. (For amount ingested, the authors state that the total doses were 5.0 g, 8.9 g, and 15.1 g, respectively; this would lead to slightly different estimates.) However, since all of the dose groups had their treatments interrupted for varying times, this figure must be adjusted to give equivalent concentrations on a continuous basis. As estimated in Figure 7-4, the numbers of weeks of treatment for the low- to high-dose groups were 76, 79, and 75.5, respectively. Multiplying these by the low- to high-dose water concentrations and dividing by 81 weeks yields 352, 731, and 1,398 ppm, respectively. Adjusting the treatment levels in the bioassay, 375 ppm in the rat would be equivalent to 352 x 2.7 = 950 ppm in the human. The other equivalent human doses are 1,974 ppm and 3,775 ppm for the middle- and high-dose groups, respectively. Assuming that a 70-kg human drinks 2 liters of water/day, the equivalent human dosages are 1.9, 3.9, and 7.6 g. Dividing by 70 kg gives doses of 27.1, 55.7, and 108.6 mg/kg/day.

The above responses on papillomas and carcinomas of the forestomach were fitted using the linearized multistage model with the equivalent human dosages.

The upper-limit maximum likelihood estimate of the linear component is

$$q_1^* = 4.7 \times 10^{-3}\ (\mathrm{mg/kg/day})^{-1}$$

Because the experiment was conducted for only 81 weeks, the adjustment factor for the less-than-natural-lifetime experiment is $(104/81)^3 = 2.1$, as discussed in a preceding section. Thus, the final value of the linear component is

$$q_1^* = 4.7 \times 10^{-3} \times 2.1 = 9.9 \times 10^{-3}\ (\mathrm{mg/kg/day})^{-1}$$

In order to estimate a unit risk for 1 ug/L of water, it was assumed that the average 70-kg human drinks 2 liters of water per day. Since 2 liters weigh approximately 2 kg, it was estimated that 1 ug/L water corresponds to 2 ug/day. Dividing by 70 kg gives 2.9×10^{-2} ug/kg/day or 2.9×10^{-5} mg/kg/body weight/day. The upper-limit unit risk corresponding to 1 ug/L epichlorohydrin concentration in water is then

$$P = 1 - \exp\ (-9.9 \times 10^{-3} \times 2.86 \times 10^{-5}) = 2.8 \times 10^{-7}$$

For comparison purposes only, the following paragraph relating the animal drinking water study and inhalation study risk estimates is included.

The dose rate d(mg/kg/day) resulting from breathing 20 m^3/day of air containing a concentration of 1 ug/m^3 can be determined if it is assumed that 100 percent of the inhaled epichlorohydrin is absorbed into the body. With this assumption the dose rate is

$$1\ \text{ug/m}^3 = 1\ \text{ug/m}^3 \times 20\ \text{m}^3\text{/day} \times 10^{-3}\ \text{mg/ug} \times 1/70\ \text{kg}$$
$$= 2.86 \times 10^{-4}\ \text{mg/kg/day}$$

The upper-limit estimate of the unit risk, P, of 1 ug/m^3 can be found using this value of d and the value of q_1^* estimated above as follows:

$$P = 1 - \exp(-9.9 \times 10^{-3} \times 2.86 \times 10^{-4}) = 2.8 \times 10^{-6}$$

This is about twice that of the animal inhalation study (see below) and about 7 percent as large as the upper limit for the human data.

In the Laskin et al. (1980) inhalation study, 15 of 140 rats exposed to short-term relatively intense exposure (100 ppm for 30 exposures) developed squamous cell carcinomas of the nasal cavity. Two others developed nasal papillomas. The same study, however, had lifetime exposure groups at the lower concentrations of 10 ppm and 30 ppm with squamous cell carcinoma incidences of 0/100 and 1/100, respectively, and no papillomas. These results are summarized in Table 7-12. The authors attempt to explain the result that dose rate rather than total dose is related to cancer incidence. This explanation is that the relationship

$$dt^n = \text{constant}$$

holds, where d is the dose rate with chronic lifetime exposure, t is the time required to reach a given level of tumor incidence, and n is a power of t, usually between 2 and 3. Thus, with increasing dose rate, not only does the incidence increase, but the time-to-tumor for a given incidence decreases. For dose rates that yield similar incidences, the time-to-tumor is greater in the

TABLE 7-12. ESTIMATION OF 95 PERCENT UPPER-LIMIT UNIT RISK ESTIMATES FOR NASAL PAPILLOMAS AND SQUAMOUS CELL CARCINOMAS USING THE MULTISTAGE MODEL WITH AND WITHOUT TIME DEPENDENCE

Model	95 percent upper-limit unit risk estimate for lifetime exposure to		Comments
	1 ppm	1 ug/m^3	
Without time dependence			
Eliminating highest dose-response group	4.8×10^{-3}	1.2×10^{-6}	Considered to be the best estimate of effects of chronic low exposures.
Including highest dose-response group as if 100 ppm given 5 days/week the entire study	4.7×10^{-3}	1.2×10^{-6}	Best estimate of linear term is zero; terms of 2nd and 3rd degree are positive.
With time dependence			
Three-stage, first stage active	4.6×10^{-2}	1.2×10^{-5}	The higher estimates show the effects of high exposure early in life.

SOURCE: Laskin et al., 1980.

lower dose rate group. In such cases, even though the incidence may not actually decrease, it may appear smaller in the lower dose rate groups because death may occur before tumorigenesis.

While the above explanation does have some experimental basis, the effects of long-term low-dose exposure are still of concern. Therefore, the low doses of 10 ppm and 30 ppm were chosen as being more representative of environmental exposures. The control groups of 100 (sham) for life and 50 untreated were combined; no squamous cell carcinomas were seen. Since epichlorohydrin was administered as a partially soluble vapor, the concentrations in ppm in experimental animals are considered equivalent to the same concentrations in humans. Thus, no corrections have been made for weight differences between species. The 95 percent upper-limit estimate for slope based on the two long-term exposures to 10 ppm and 30 ppm (1.79 ppm and 5.36 ppm continuous dose) is

$$q_h^* = 4.8 \times 10^{-3} \text{ (ppm)}^{-1}$$

For unit risk in terms of ug/m^3, we make the transformation

$$1 \text{ ug/m}^3 = \frac{10^{-3} \text{ ppm}}{1.2 \text{ (m.w. chemicals)/(m.w. air)}} = \frac{10^{-3} \text{ ppm}}{1.2\ (92.5)/(28.8)}$$

$$= 2.59 \times 10^{-4} \text{ ppm}$$

thus, in terms of ug/m^3

$$q_h^* = 4.8 \times 10^{-3} \text{ (ppm)}^{-1} \times \frac{2.59 \times 10^{-4} \text{ ppm}}{1 \text{ ug/m}^3} = 1.2 \times 10^{-6} \text{ (ug/m}^3)^{-1}$$

For comparison purposes, the above data were extrapolated to low-dose estimates using the nasal papilloma and squamous cell carcinoma responses to the 30-exposure 100-ppm dose as though the dose had been given for the animals' lifetimes. The results produced an upper-limit estimate of $q_1^* = 4.7 \times 10^{-3}$, but with higher-order terms of the second and third degree. These are shown in Table 7-12. Also shown in Table 7-12 are the results based on the partial lifetime exposure model, computed with the ADOLL1-83 computer program (Crump and Howe, 1983, 1984). The partial lifetime exposure model is one which is linear in dose but one in which the time-to-tumor (or time-to-tumor death) variable has an exponent related to both the number of stages and the stage affected by the carcinogen (single active stage). For the epichlorohydrin data, all animals with nasal papillomas (2) or carcinomas (15) were considered to have died as a result of these tumors. This approach is conceptually consistent with that of the above analysis of the quantal data which counted the number of animals with tumors. It is also consistent with the study design, which did not include scheduled sacrifice, so that all animals sacrificed were killed in moribund condition. Finally, the data often note times at which the tumors were first observed; these times were close to the actual death times. The result of ascertaining that all animals with nasal carcinomas died from them is that the time-to-tumor death variable can present a good time-response function. Based on the method of maximum likelihood, the best-fitting model is one with three stages, with the first stage active. Satisfactory fits were also obtained with models of stages 4, 5, and 6, all with stage 1 active. The results show that accounting for partial lifetime exposure leads to a 95 percent upper-limit estimate of the lifetime unit risk of 4.6×10^{-2} $(\text{ppm})^{-1}$, which is almost 10 times that derived when partial exposure is not accounted for.

The results of the above analysis differ from those of most other EPA analyses in that they show how the response is related to dose rate in a nonlinear way. While the design of the study could not detect whether epichlorohydrin given at high doses later in life would be a late-stage carcinogen, the model was very definitely able to detect the danger of high doses given early. The implication for humans is that high exposures to epichlorohydrin early in life for a short time can be more hazardous than larger cumulative doses given over a longer period. Whether or not that same high dose given later in life would be as hazardous, the study cannot answer. The fact that only an early-stage active carcinogen model fits the data, however, is consistent with the observed response.

7.1.4.2.3 Summary of Unit Risk Estimates. Three upper-limit risk estimates were calculated for epichlorohydrin. All three have more uncertainty than those of other suspect carcinogens that the CAG has evaluated. Epichlorohydrin is, however, among the weakest of these in terms of unit risks.

Quantitative unit risks were calculated for epichlorohydrin via both the drinking water and the inhalation routes. The study of male Wistar rats (Konishi et al., 1980) was used to estimate a unit risk, 2.8×10^{-7}, for a lifetime exposure to drinking water containing 1 ug/L of epichlorohydrin. This estimate has the uncertainty of estimated exposure to the target organ, the small number of animals used in the study, and the high mortality that was observed.

Quantitative risk assessments were also calculated using both the rat inhalation study (Laskin et al., 1980) and the study of Shell Oil workers (Enterline, 1981). The two 95 percent upper-limit unit risk estimates are not very close: the upper limit for animal data is 1.2×10^{-6} $(ug/m^3)^{-1}$; the upper-limit for human data is 3.9×10^{-5} $(ug/m^3)^{-1}$. In units of risks per ppm, the upper-limit estimates range from 4.8×10^{-3} to 4.6×10^{-2} $(ppm)^{-1}$ for animals and 0.153 $(ppm)^{-1}$ for humans. In view of the weakness of both inhalation data bases, these inhalation unit risk estimates must be taken with caution. Animal exposures in the Laskin et al. (1980) inhalation study, on a continuous daily equivalent basis, were 0.86 ppm, 2.98 ppm, and 1.03 ppm for actual daily exposures of 10 ppm, 30 ppm, and 100 ppm, respectively. The high concentration, 100 ppm, was given for only 30 exposures, whereas the lower two concentrations were given for the animals' lifetimes; however, only this high-concentration group developed a significant increase in cancers. Because of the dose-rate effect and/or the early active stage effect, each estimate based on the animal inhalation study should extrapolate to the specific human exposure condition. The estimate $q_1^* = 4.8 \times 10^{-3}$ $(\text{ppm})^{-1}$ should be used for long-term low exposures; the estimate $q_1^* = 4.6 \times 10^{-2}$ should be used under conditions of short-term high exposure. For human-to-human extrapolations, the 95 percent upper-limit unit risk estimate based on human studies is also an upper bound on non-statistically-significant increases in cancer mortality.

7.1.4.3 Relative Potency--One of the uses of unit risk is to compare the potency of carcinogens. To estimate the relative potency, the unit risk slope factor is multiplied by the molecular weights, and the resulting number is expressed in terms of $(\text{mmol/kg/day})^{-1}$. This is called the relative potency index.

Figure 7-7 is a histogram representing the frequency distribution of potency indices of 53 suspect carcinogens evaluated by the CAG. The actual data summarized by the histogram are presented in Table 7-13. Where positive human data are available for a compound, they have been used to calculate the index. Where no human data are available, animal oral studies and animal inhalation studies have been used, in that order. In the present case, the human data are only suggestive; therefore, animal oral studies were used.

The potency index for epichlorohydrin based on the drinking water study (Konishi et al., 1980; Kawabata, 1981) is 0.92 $(\text{mmol/kg/day})^{-1}$. This is derived

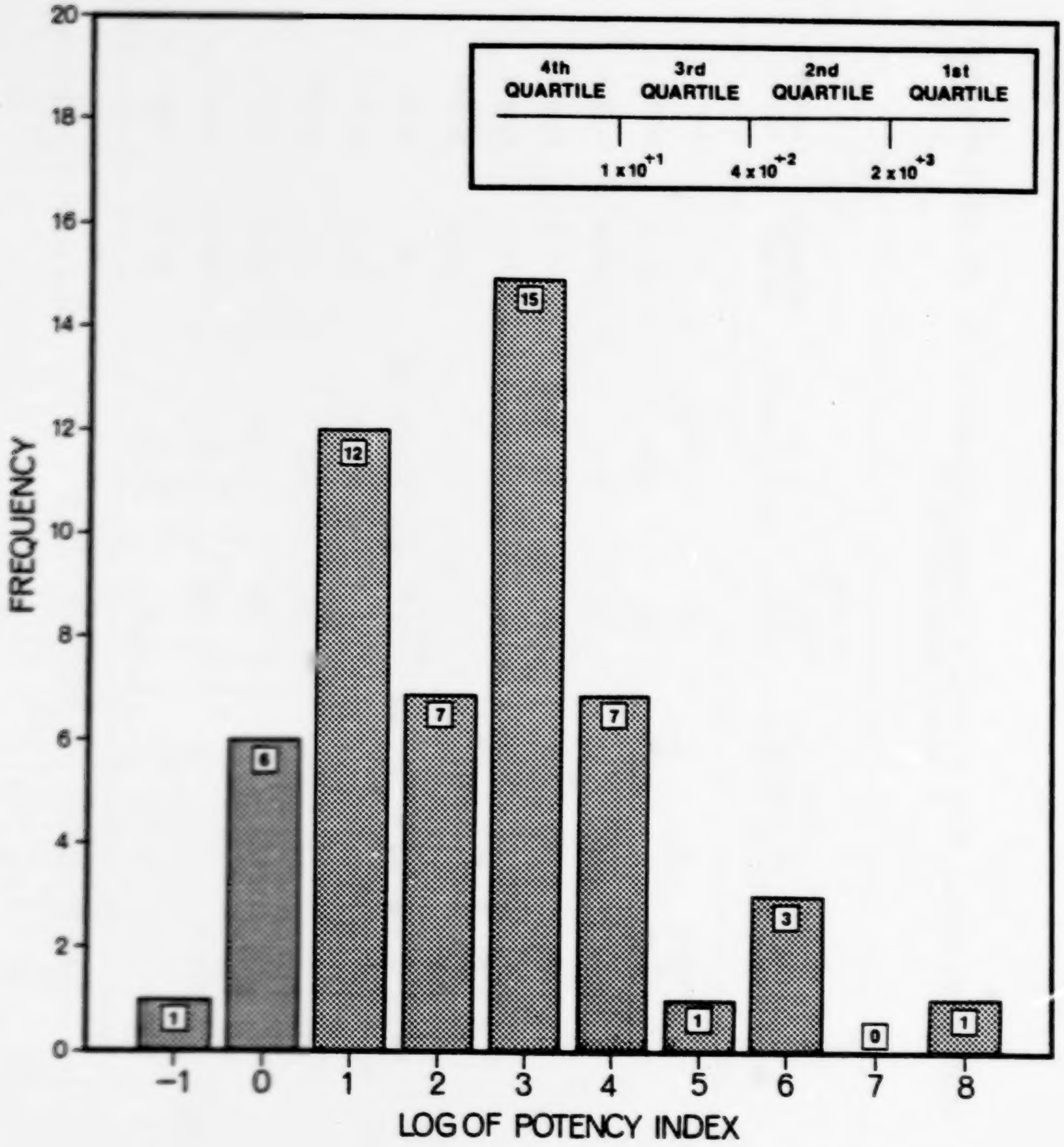

Figure 7-7. Histogram representing frequency distribution of the potency indices of 53 suspect carcinogens evaluated by the Carcinogen Assessment Group.

TABLE 7-13. RELATIVE CARCINOGENIC POTENCIES AMONG 53 CHEMICALS EVALUATED BY THE CARCINOGEN ASSESSMENT GROUP AS SUSPECT HUMAN CARCINOGENS

Compounds	CAS Number	Level of evidence[a]		Grouping based on IARC criteria	Slope $(mg/kg/day)^{-1}$	Molecular weight	Potency index	Order of magnitude ($\log_{10}$ index)
		Humans	Animals					
Acrylonitrile	107-13-1	L	S	2A	0.24(W)	53.1	$1x10^{+1}$	+1
Aflatoxin B_1	1162-65-8	L	S	2A	2900	312.3	$9x10^{+5}$	+6
Aldrin	309-00-2	I	L	2B	11.4	369.4	$4x10^{+3}$	+4
Allyl chloride					$1.19x10^{-2}$	76.5	$9x10^{-1}$	0
Arsenic	7440-38-2	S	I	1	15(H)	149.8	$2x10^{+3}$	+3
B[a]P	50-32-8	I	S	2B	11.5	252.3	$3x10^{+3}$	+3
Benzene	71-43-2	S	S	1	$5.2x10^{-2}$(W)	78	$4x10^{0}$	+1
Benzidene	92-87-5	S	S	1	234(W)	184.2	$4x10^{+4}$	+5
Beryllium	7440-41-7	L	S	2A	2.6	9	$2x10^{+1}$	+1
Cadmium	7440-43-9	L	S	2A	7.8(W)	112.4	$9x10^{+2}$	+3
Carbon tetrachloride	56-23-5	I	S	2B	$1.30x10^{-1}$	153.8	$2x10^{+1}$	+1
Chlordane	57-74-9	I	L	3	1.61	409.8	$7x10^{+2}$	+3

[a] S = Sufficient evidence; L = Limited evidence; I = Inadequate evidence.

(continued on the following page)

TABLE 7-13. (continued)

Compounds	CAS Number	Level of evidence[a]		Grouping based on IARC criteria	Slope (mg/kg/day)$^{-1}$	Molecular weight	Potency index	Order of magnitude (log$_{10}$ index)
		Humans	Animals					
Chlorinated ethanes								
1,2-dichloroethane	107-06-2	I	S	2B	6.9x10^{-2}	98.9	7x10^{0}	+1
hexachloroethane	67-72-1	I	L	3	1.42x10^{-2}	236.7	3x10^{0}	0
1,1,2,2-tetrachloroethane	79-34-5	I	L	3	0.20	167.9	3x10^{+1}	+1
1,1,2-trichloroethane	79-00-5	I	L	3	5.73x10^{-2}	133.4	8x10^{0}	+1
Chloroform	67-66-3	I	S	2B	7x10^{-2}	119.4	8x10^{0}	+1
Chromium (VI)	7440-47-3	S	S	1	41(W)	100	4x10^{+3}	+4
DDT	50-29-3	I	S	2B	0.34	354.5	1x10^{+2}	+2
Dichlorobenzidine	91-94-1	I	S	2B	1.69	253.1	4x10^{+2}	+3
1,1-dichloroethylene	75-35-4	I	L	3	1.47x10^{-1}(I)	97	1x10^{+1}	+1
Dieldrin	60-57-1	I	S	2B	30.4	380.9	1x10^{+4}	+4
2,4-dinitrotoluene	121-14-2	I	S	2B	0.31	182	6x10^{+1}	+2
Diphenylhydrazine	122-66-7	I	S	2B	0.77	180	1x10^{+2}	+2
Epichlorohydrin	106-89-8	I	S	2B	9.9x10^{-3}	92.5	9x10^{-1}	0
Bis(2-chloroethyl)ether	111-44-4	I	S	2B	1.14	143	2x10^{+2}	+2
Bis(chloromethyl)ether	542-88-1	S	S	1	9300(I)	115	1x10^{+6}	+6

[a] S = Sufficient evidence; L = Limited evidence; I = Inadequate evidence.

(continued on the following page)

TABLE 7-13. (continued)

Compounds	CAS Number	Level of evidence[a] Humans	Level of evidence[a] Animals	Grouping based on IARC criteria	Slope $(mg/kg/day)^{-1}$	Molecular weight	Potency index	Order of magnitude ($\log_{10}$ index)
Ethylene dibromide (EDB)	106-93-4	I	S	2B	41	187.9	$8\times10^{+3}$	+4
Ethylene oxide	75-21-8	L	L	2A	1.26(I)	44.1	$6\times10^{+1}$	+2
Heptachlor	76-44-8	I	S	2B	3.37	373.3	$1\times10^{+3}$	+3
Hexachlorobenzene	118-74-1	I	S	2B	1.67	284.4	$5\times10^{+2}$	+3
Hexachlorobutadiene	87-68-3	I	L	3	7.75×10^{-2}	261	$2\times10^{+1}$	+1
Hexachlorocyclohexane								
technical grade					4.75	290.9	$1\times10^{+3}$	+3
alpha isomer	319-84-6	I	S	2B	11.12	290.9	$3\times10^{+3}$	+3
beta isomer	319-85-7	I	L	3	1.84	290.9	$5\times10^{+2}$	+3
gamma isomer	58-89-9	I	L	2B	1.33	290.9	$4\times10^{+2}$	+3
Hexachlorodibenzodioxin	34465-46-8	I	S	2B	$6.2\times10^{+3}$	391	$2\times10^{+6}$	+6
Methylene chloride	75-09-2	I	L	3	6.3×10^{-4}	84.9	5×10^{-2}	-1
Nickel	7440-02-0	L	S	2A	1.15(W)	58.7	$7\times10^{+1}$	+2
Nitrosamines								
Dimethylnitrosamine	62-75-9	I	S	2B	25.9(not by q_1^*)	74.1	$2\times10^{+3}$	+3
Diethylnitrosamine	55-18-5	I	S	2B	43.5(not by q_1^*)	102.1	$4\times10^{+3}$	+4
Dibutylnitrosamine	924-16-3	I	S	2B	5.43	158.2	$9\times10^{+2}$	+3

[a] S = Sufficient evidence; L = Limited evidence; I = Inadequate evidence.

(continued on the following page)

TABLE 7-13. (continued)

Compounds	CAS Number	Level of evidence[a] Humans	Level of evidence[a] Animals	Grouping based on IARC criteria	Slope $(mg/kg/day)^{-1}$	Molecular weight	Potency index	Order of magnitude ($\log_{10}$ index)
N-nitrosopyrrolidine	930-55-2	I	S	2B	2.13	100.2	$2\times10^{+2}$	+2
N-nitroso-N-ethylurea	759-73-9	I	S	2B	32.9	117.1	$4\times10^{+3}$	+4
N-nitroso-N-methylurea	684-93-5	I	S	2B	302.6	103.1	$3\times10^{+4}$	+4
N-nitroso-diphenylamine	86-30-6	I	S	2B	4.92×10^{-3}	198	1×10^{0}	0
PCBs	1336-36-3	I	S	2B	4.34	324	$1\times10^{+3}$	+3
Phenols								
2,4,6-trichlorophenol	88-06-2	I	S	2B	1.99×10^{-2}	197.4	4×10^{0}	+1
Tetrachlorodibenzo-p-dioxin (TCDD)	1746-01-6	I	S	2B	$1.56\times10^{+5}$	322	$5\times10^{+7}$	+8
Tetrachloroethylene	127-18-4	I	L	3	3.5×10^{-2}	165.8	6×10^{0}	+1
Toxaphene	8001-35-2	I	S	2B	1.13	414	$5\times10^{+2}$	+3
Trichloroethylene	79-01-6	I	L	3	1.9×10^{-2}	131.4	2.5×10^{0}	0
Vinyl chloride	75-01-4	S	S	1	1.75×10^{-2}(I)	62.5	1×10^{0}	0

[a] S = Sufficient evidence; L = Limited evidence; I = Inadequate evidence.

Remarks:

1. Animal slopes are 95% upper-limit slopes based on the linearized multistage model. They are calculated based on animal oral studies, except for those indicated by I (animal inhalation), W (human occupational exposure), and H (human drinking water exposure). Human slopes are point estimates based on the linear nonthreshold model.

2. The potency index is a rounded-off slope in $(mmol/kg/day)^{-1}$ and is calculated by multiplying the slopes in $(mg/kg/day)^{-1}$ by the molecular weight of the compound.

3. Not all of the carcinogenic potencies presented in this table represent the same degree of certainty. All are subject to change as new evidence becomes available.

as follows: the upper-limit slope estimate from the drinking water study is 9.9×10^{-3} (mg/kg/day)$^{-1}$. Multiplying by the molecular weight of 92.5 gives a potency index of 9.2×10^{-1}. Rounding off to the nearest order of magnitude gives a value of 10^0, which is the scale presented on the horizontal axis of Figure 7-7. The index of 0.92 lies in the fourth quartile of the 53 suspect carcinogens that the CAG has evaluated, placing epichlorohydrin among the weakest of these carcinogens.

7.1.5 Summary

7.1.5.1 Qualitative Assessment--The carcinogenicity of epichlorohydrin has been demonstrated in rats and mice. Epichlorohydrin vapor produced papillomas and squamous cell carcinomas in the nasal tracts of male Sprague-Dawley rats initially given 30 daily exposures, 6 hours/day, followed by lifetime observation. Consumption of epichlorohydrin in drinking water elicited neoplastic lesions in the forestomachs of male Wistar rats, including a statistically significant increase in the combined incidence of papillomas and carcinomas in high-dose animals. The drinking water study is compromised in that pathologic evaluations of decedents were not reported due to postmortem changes, the 81-week duration of the study was less than the lifetimes of the animals, and the number of animals in each dosage group was small. In a draft report of a study in which epichlorohydrin was administered in water via gavage, a strong dose-response with forestomach papillomas and carcinomas was present in both male and female Wistar rats. This study presented strong qualitative evidence on the proximal-site carcinogenicity of epichlorohydrin.

Two studies involving dermal application of epichlorohydrin on the skin of mice for their lifetimes elicited no tumor response. In one, thrice weekly applications at a maximally tolerated dose were given to ICR/Ha mice, and in the other, an uncertain dose (i.e., one brushful) was applied three times weekly to the skin of C3H mice. On the basis of these results, epichlorohydrin is apparently ineffective as a complete carcinogen when applied to the skin. Skin tumor initiating activity was found in a lifetime initiation-promotion study with female ICR/Ha mice.

Weekly subcutaneous injection of epichlorohydrin at a maximally tolerated dose in a lifetime study in female ICR/Ha mice produced a statistically significant increase in local sarcomas. However, intraperitoneal injections once weekly in females of this strain proved ineffective. None of the above studies showed evidence of metastases of the proximal-site tumors.

A single subcutaneous injection of a low dose of epichlorohydrin did not produce a carcinogenic effect in a lifetime observation study with C3H mice; however, survival in this study was poor, and the single low dose used would appear to be a relatively weak challenge compared to lifetime treatment with doses as high as those maximally tolerated.

Three epidemiologic studies of mortality in epichlorohydrin workers have been conducted. One study of epichlorohydrin workers at Dow Chemical Company in Texas failed to show an increase in cancer. This study is considered inadequate for the evaluation of carcinogenicity, however, due to low exposure, short exposure duration, short latency period, and young age of the cohort. A second study of European epichlorohydrin workers at plants in four different countries also showed no association between epichlorohydrin and cancer, but was also judged to be inadequate for many of the same reasons as the Dow study.

A third study, a 1979 update of an ongoing study of workers at Shell Oil Company, showed increased deaths from respiratory cancer. Leukemia was also present in an otherwise healthy cohort. This increase, however, was not statistically significant, and the trend in the most recent 2-year follow-up period actually weakened the evidence that epichlorohydrin is a human carcinogen.

While the previous update had showed increasing lung cancer trends with time since first exposure, this most recent update produced only one additional cancer death and no additional lung cancer deaths.

7.1.5.2 Quantitative Assessment--Unit risk estimates for exposure to epichlorohydrin have been calculated by the CAG for both the animal and human studies. For the animal studies, unit risk estimates were calculated from both drinking water and inhalation studies. The study of male Wistar rats exposed to epichlorohydrin in drinking water indicated that epichlorohydrin caused tumors of the forestomach. Based on this study, the CAG estimated 2.8×10^{-7} as the upper-limit lifetime risk from exposure to drinking water containing 1 ug/L of epichlorohydrin. For the animal inhalation studies, a unit risk was calculated using the nasal carcinoma response in male Sprague-Dawley rats exposed to epichlorohydrin vapor in the Laskin et al. (1980) study. In this study, the 10 percent nasal carcinoma response at the 100 ppm exposure level was not used for low-level environmental exposure extrapolation because it is a high-dose short-term exposure and is not consistent with the low-dose long-term exposure of the other groups. In order to extrapolate to low environmental levels, the CAG used the multistage model on the controls and the two lower doses to provide a 95 percent upper limit of risk. The linearized multistage model was used for low-dose extrapolation in order to give an upper-bound estimate of lifetime cancer risk, recognizing that uncertainties in both the qualitative evaluation and the quantitative extrapolation method can yield a lower bound of risk approaching zero. The plausibility of the upper bound is enhanced when there is sufficient evidence for genotoxicity, which is the case with epichlorohydrin.

Using this procedure, the plausible upper bound of the individual lifetime cancer risk resulting from continuous exposure to air with an epichlorohydrin level of 1 ug/m^3 is 1.2×10^{-6}. For high exposures of short duration early in life, the same study, using the 100 ppm exposure level, estimates lifetime risks ten times as high.

The study of employees from the Shell Oil Company was used to provide a unit risk estimate for exposure to epichlorohydrin based on human epidemiologic data. This study showed an increase in respiratory cancer deaths in an otherwise healthy population. While this increase was not statistically significant, the evidence for the carcinogenicity of epichlorohydrin in animals suggests that a comparison of estimated risk levels should be made. On the basis of these human data, the 95 percent upper-limit cancer risk resulting from continuous exposure to air with an epichlorohydrin level of 1 ug/m^3 is estimated to be 3.9×10^{-5}. Since this estimate, which is based on a nonsignificant increase, is higher than that from the animal inhalation study, the lower animal-based estimate of 1.2×10^{-6} $(ug/m^3)^{-1}$ will be used as the 95 percent upper-limit unit risk estimate for inhalation exposure.

7.1.6 Conclusions

The animal evidence for the carcinogenicity of epichlorohydrin includes nasal carcinomas in rats, local sarcomas in mice, and forestomach neoplasms in rats. Applying the International Agency for Research on Cancer (IARC) classification system (Appendix F), this level of evidence would be considered sufficient for concluding that epichlorohydrin is carcinogenic in experimental animals. Although the human epidemiologic evidence with regard to epichlorohydrin alone is negative, sequential exposure to the IPA process and epichlorohydrin produced evidence for the carcinogenicity of epichlorohydrin. However, this evidence has only marginal statistical significance ($P < 0.1$). Using the IARC system for describing the overall evidence for carcinogenicity, epichlorohydrin would be classed as a 2B chemical.

As described in the mutagenicity section, epichlorohydrin has been demonstrated to be mutagenic in both prokaryotic and eukaryotic systems. Epichlorohydrin is a direct-acting alkylating agent, and therefore does not require metabolic activation to attack biological macromolecules.

Quantitative estimates of the carcinogenic potency of epichlorohydrin have been made by the CAG for both drinking water and inhalation. On the basis of forestomach tumors in male Wistar rats exposed to epichlorohydrin in drinking water, a lifetime exposure to 1 ug/L of epichlorohydrin in drinking water is estimated to present an upper-limit risk of 2.8×10^{-7}.

The CAG's inhalation estimates were made on the basis of animal data on nasal carcinomas and human data on a non-statistically significant increase in respiratory cancers. These two unit risk estimates are not very close; the upper-limit estimate from nonsignificant human data is 3.9×10^{-5} $(ug/m^3)^{-1}$; the upper-limit estimate from positive animal data is 1.2×10^{-6} $(ug/m^3)^{-1}$. The estimate from the animal data is chosen. The estimate based on the nonsignificant increase in the human data based would have been chosen only if it had been lower than the animal-based estimate. The animal data estimate does not use the nasal carcinoma response to short-term high exposures because the CAG feels that such an exposure does not reflect environmental experience and is not consistent with the long-term lower-dose response. Under conditions of short-term high exposures, lifetime risks can be extrapolated and are estimated to be greater than from long-term exposures by a factor of ten.

The carcinogenic potency of epichlorohydrin lies in the fourth quartile among the 53 suspect carcinogens evaluated by the CAG. This places epichlorohydrin among the weakest of the substances that the CAG has evaluated as suspect carcinogens.

7.2 MUTAGENICITY

7.2.1 Introduction

Chemicals that induce gene mutations and chromosomal aberrations have been regarded as a potential risk to human health. If mutations occur in human germ cells, they may be passed on to future generations, causing deleterious effects. On the other hand, if mutations occur in somatic cells, they may lead to the onset of diseases such as cancer. The aim of mutagenicity risk determination is to assess the risk that particular chemicals pose to human well-being.

Epichlorohydrin has been tested for its ability to cause genetic damage in both prokaryotic and eukaryotic systems. The prokaryotic systems include assays for gene mutations and reparable genetic damage in bacteria. The eukaryotic systems include gene mutation studies in yeast, *Drosophila*, and mammalian cells, and chromosomal aberration studies in human and other mammalian cells exposed to epichlorohydrin both in vitro and in vivo. Positive findings in most of these mutagenicity assays clearly indicate that epichlorohydrin is a mutagen. The following is an analysis of the literature pertaining to the mutagenic effects of epichlorohydrin.

7.2.2 Gene Mutations in Bacteria

7.2.2.1 *Salmonella Assay*--The potential of epichlorohydrin to induce reverse mutations in *Salmonella typhimurium* has been documented by many investigators. Sram et al. (1976) tested epichlorohydrin for the induction of back mutations (revertants) in *S. typhimurium* using both the spot test and suspension assay. In the spot test, *S. typhimurium* strains *his*G46, TA100, TA1950, TA1951, TA1952, TA1534, TA1537, and TA1538 were used. Epichlorohydrin (purity not given) concentrations of 1 percent (0.05 μmole), 5 percent (0.27 μmole), 10 percent (0.54 μmole), and 100 percent (1.10 μmole) were employed. Epichlorohydrin was applied in 50 μl quantities on to the center of the bacterial petri dishes. Positive (+) results were noted only in the strains *his*G46 and TA100. The positive results with *his*G46 and TA100 together with the negative result with the other strains indicate that epichlorohydrin is acting as a base-pair substitution mutagen in *Salmonella*. Furthermore, epichlorohydrin is active without metabolism by mammalian enzymes and that this is consistent with its known activity as an alkylating agent and ability to react directly with DNA bases (Sram et al., 1981).

In the suspension assay, only strains his G46 and TA100 were used. The cells of the strain his G46 were treated with epichlorohydrin at concentrations of 1.08×10^{-4}, 1.08×10^{-3}, 5.40×10^{-3}, 1.08×10^{-2}, 2.70×10^{-2}, 5.40×10^{-2}, 1.08×10^{-1}, and 5.40×10^{-1} M for 60 minutes without a metabolic activation system (S-9) and assayed for revertant colonies. The concentration of 5.40×10^{-1} M was toxic and produced 100 percent cell killing. Numbers of revertants/10^{9} survivors were 6, 4, 9, 18, 15, 1.68×10^{3}, 3.18×10^{7} for the above concentrations, respectively. In TA100, epichlorohydrin concentrations of 1.08×10^{3}, 5.40×10^{-3}, 1.08×10^{-2}, 5.40×10^{-2}, and 1.08×10^{-1} M were used. The revertant frequencies obtained respectively for these concentrations, except for the concentration 1.08×10^{-1} M, which was toxic for 100 percent of the cells, were 2.25×10^{10}, 9.64×10^{1}, 2.85×10^{5}, 3.44×10^{5}, and 5.00×10^{6}. The spontaneous revertant frequencies were 6 in his G46 and 2.25×10^{1} in TA100. A clear dose-response relationship was evident in the experimental groups. These results indicate that epichlorohydrin is mutagenic in S. typhimurium strains his G46 and TA100.

Andersen et al. (1978) tested epichlorohydrin for its mutagenic potential in the plate incorporation assay using S. typhimurium strains TA100 and TA1535 as described by Ames et al. (1975). In strain TA100, epichlorohydrin doses of 0.5, 1.0, 1.5, 2.0, and 2.5 µmoles/plate (in 100 µl DMSO) gave 352, 500, 650, 800, and 1,200 revertants/plate, respectively, indicating a clear dose-response relationship (Figure 7-8). The experiment was carried out in the absence of an S-9 mix. The spontaneous frequency of revertants for TA100 was 250 revertants/plate. No solvent control data were given in this paper. In strain TA1535, the number of revertants/plate were 216, 474, and 1,418 at epichlorohydrin concentrations of 0.254, 0.635, and 2.54 µmoles/plate in the absence of an S-9 mix. In the presence of an S-9 mix, doses of 2.54, 6.35, and 25.4 µmoles/plate induced 34, 152, and 911 revertants/plate, respectively. The spontaneous frequency in the strain TA1535 was 36 revertants/plate. These results indicate that epichlorohydrin is mutagenic in Salmonella strains TA100 and TA1535.

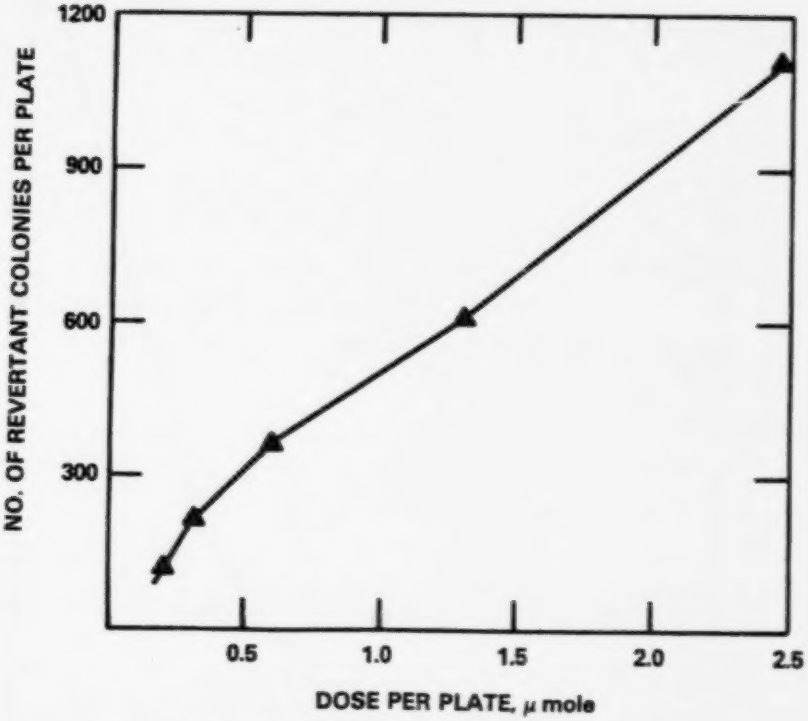

Figure 7-8. Mutagenicity of epichlorohydrin for *S. typhimurium* TA100 (revert by base-pair substitution).

Source: Andersen et al. (1978).

Planche et al. (1979) also investigated the mutagenic potential of epichlorohydrin in S. typhimurium strain TA100 using the plate incorporation assay (Ames et al. 1975). Epichlorohydrin (Merck-Schuchardt, Darmstadt, F.R.G.) concentrations of 10, 100, 1,000, and 5,000 μmoles in 0.1 ml actone/plate were employed in the study. Epichlorohydrin was mutagenic with a clear dose-response relationship (Figure 7-9).

Stolzenberg and Hine (1979) also detected positive results with a clear dose-response relationship in S. typhimurium strains TA100 and TA1535 using epichlorohydrin (purity 99+%, Aldrich Chemicals) concentrations of 2, 4, 6, 8, and 10 μmoles/plate both in the presence and absence of an S-9 mix (Figure 7-10).

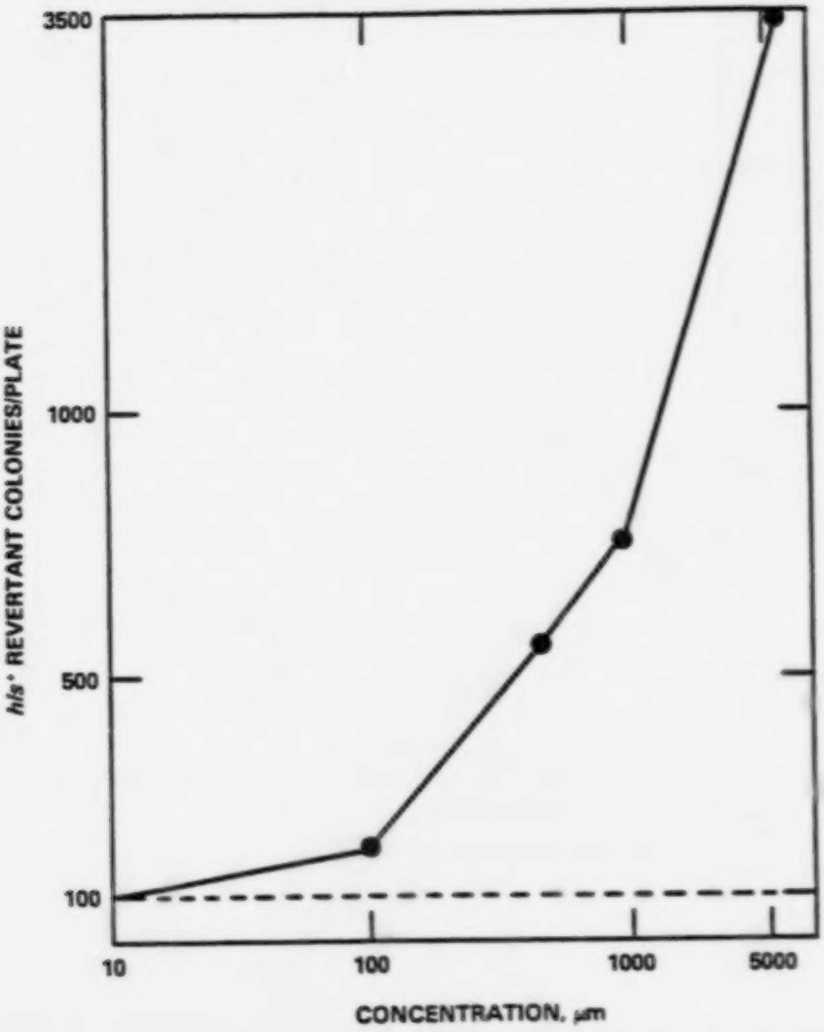

Figure 7-9. Mutagenicity of epichlorohydrin (●), at various concentrations (nmol/ml of soft agar) in *S. typhimurium* TA100[a].

[a]The compounds were added as an acetone solution 0.1 ml/plate). Solvent control assays (– – – – –). The number of spontaneous *his* revertants/plate has not been subtracted. Mean values from 3 to 6 plates are plotted.

Source: Planche et al. (1979).

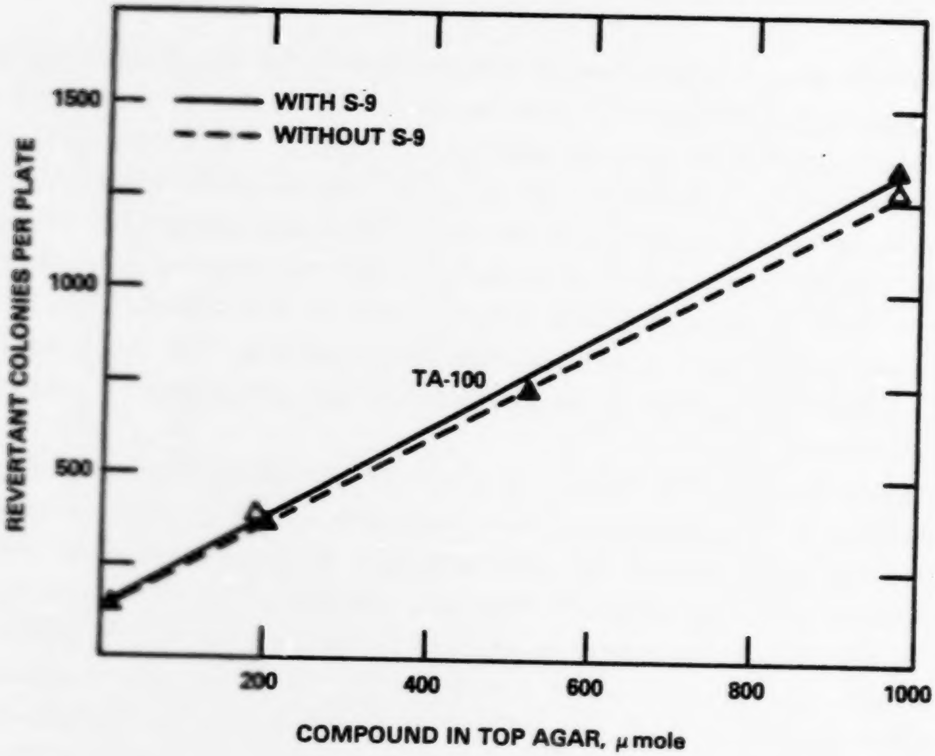

Figure 7-10. Dose-response curves for epichlorohydrin.

Source: Stolzenberg and Hine (1979).

Eder et al. (1980) investigated the mutagenic potential of epichlorohydrin in the *S. typhimurium* strain TA100 both in the presence and absence of S-9 mix using the suspension assay. Epichlorohydrin (purity 99.5%) induced 275 revertants/µmoles and in the presence of S-9 mix there were 70 revertants/ umoles. There was a clear linear dose-response relationship between the number of revertants/plate and the concentrations of the test compound (Figure 7-11).

Bartsch et al. (1980), tested epichlorohydrin for its mutagenic potential in *S. typhimurium* strains TA100 and TA1535 in the absence of an S-9 mix using the plate incorporation assay of Ames et al. (1975). These investigators used an epichlorohydrin concentration of 1.1×10^{-2} µmoles/plate and found epichlorohydrin to be highly mutagenic in the strain TA100 and mutagenic in the strain TA1535. These investigators also mention that the dose-response curve is linear. Detailed tabulated data are not given in this report. Based on the other reports available on epichlorohydrin mutagenicity, the report of Bartsch et al. (1980) is regarded as an indication of positive mutagenic response of epichlorohydrin.

Elmore et al. (1976) and Voogd et al. (1981) detected epichlorohydrin to be mutagenic in *S. typhimurium* strain TA100 with a dose-response relationship. However, the test compound was more mutagenic in the absence of an S-9 mix.

Bridges (1978) detected 600 revertant colonies/plate with a concentration of 2 µg/ml of epichlorohydrin in agar in *S. typhimurium* strain TA1535 when plates were incubated in sealed airtight jars. When the same concentration was added externally to the plates and allowed to evaporate into the sealed jar, only 300 colonies per plate were induced. This indicates that epichlorohydrin can freely penetrate into aqueous media. If agar plates containing epichlorohydrin are not incubated in a sealed incubator, the activity may be lost as indicated by fewer induced mutants. Simmon, as quoted by Bridges (1978), was able to detect revertants in *S. typhimurium* strain TA1535 at a concentration of 3 µg/l air, which happens to be the U.S. OSHA maximally allowed concentration for a 10-hour occupational exposure period. In strain TA100, Bridges (1978) detected that a concentration of 1.25 µg/l air to be mutagenic when the plates were incubated in sealed airtight containers.

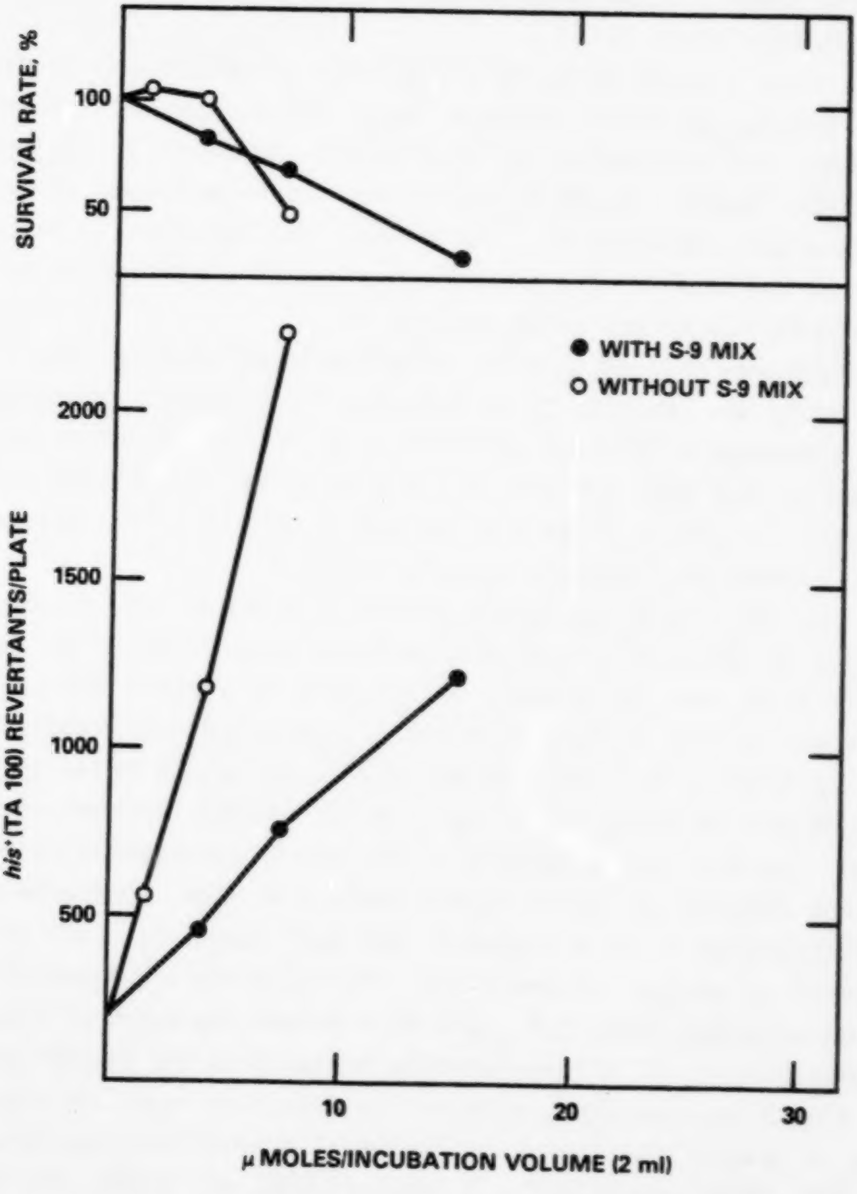

Figure 7-11. Mutagenicity of epichlorohydrin with (●) and without (○) S-9 mix.

Source: Eder et al. (1980).

Laumbach et al. (1977) reported epichlorohydrin to be mutagenic for S. typhimurium strain TA100. Epichlorohydrin at a concentration of 4.746 µmoles/plate induced 2,856 revertants/plate. No dose-response data were available in this report.

It is clear from the foregoing account that epichlorohydrin is mutagenic in the S. typhimurium reverse mutation assay with a clear-cut dose-response relationship. Epichlorohydrin was particularly active in S. typhimurium strains TA100, TA1535, and G46 indicating that it is an inducer of mutations through base-pair substitution. Furthermore, the mutagenic activity is expressed in the absence of a metabolic activation system indicating that epichlorohydrin is a direct-acting mutagen.

7.2.2.2 Mutations in Klebsiella--In an abstract published by Voogd (1973), epichlorohydrin was reported to be mutagenic in Klebsiella pneumoniae by inducing streptomycin-resistant mutations. No details regarding the concentrations of the test compound or the frequencies of mutations in the experimental and control groups are provided in this abstract. Consequently, this report cannot be critically evaluated.

7.2.2.3 Host-Mediated Assay--Epichlorohydrin was tested for its ability to induce reverse mutations in the host-mediated assay (Sram et al. 1976). Female ICR mice, aged 10-12 weeks and weighing 35 g each, were injected intraperitoneally with tester strains of S. typhimurium G46, TA100, TA1950, TA1951, and TA1952. The test compound (purity not given) in the concentrations of 50 and 100 mg/kg, dissolved in 0.2 ml of DMSO, was administered to groups of five mice intramuscularly. The control group consisted of five animals and received the tester strains and 0.2 ml DMSO. Mice were killed 3 hours postinjection of epichlorohydrin, and their peritoneal fluid containing the bacteria was assayed for revertants. The result was expressed as C, which is a relative mutagenicity; i.e., the ratio between the mutation frequency in the experimental groups and the mutation frequency in the control group. C greater than 2 was considered to be a significant increase. A significant increase (C greater than 2) in the frequency of revertants was noted for strains G46, TA100, and TA1950. In strains TA1951 and TA1952, the revertant frequency was similar to the control frequency (C less than 2). These results indicate that epichlorohydrin is mutagenic in the host-mediated assay employing Salmonella strains G46, TA100, and TA1950.

7.2.2.4 Body Fluid Analysis--In an abstract published by Kilian et al. (1978), urine samples of two industrial workers exposed to epichlorohydrin (25 ppm), as a result of accidental spill, induced a twofold increase in the revertant frequency in *S. typhimurium* TA1535 over the control value. In six workers exposed to low levels (0.8-4.0 ppm) of epichlorohydrin, the urine samples showed no increase in the revertant frequency over the control value. Urine samples of mice orally exposed to 200-400 mg/kg of epichlorohydrin also exhibited mutagenic activity in *S. typhimurium* TA1535. This abstract contained no information on the number of revertants in the experimental and control groups and thus critical evaluation cannot be made.

7.2.3 Bacterial DNA Repair Tests

Epichlorohydrin was tested for its ability to damage the DNA using the PolA plate assay of Rosenkranz and the Rec-assay of Kada (Bridges 1981: Elmore et al. 1976). These tests revealed that epichlorohydrin produced reparable DNA damage similar to that of an alkylating agent in the absence of metabolic activation. Epichlorohydrin at a concentration of 0.01 µg/ml produced a positive response in the PolA assay. In the Rec-assay, a concentration of 0.1 ug/ml produced a positive result.

7.2.4 Gene Mutations in Neurospora

Epichlorohydrin was tested for its ability to induce point mutations (reverse mutations) in the mold *Neurospora crassa* (Kolmark and Giles 1955). The purple adenineless mutant, 38701 strain, of *N. crassa* was used in this experiment.

Epichlorohydrin at a concentration of 0.15 M (14 mg/ml) was added to the suspension of microconidia at 25°C and allowed to incubate for 15, 30, 45, and 60 minutes. The microconidia were washed free of the test compound and plated on minimal agar plates. The number of viable and of surviving conidia in the treated and control series was determined by plating diluted samples on minimal medium supplemented with adenine. The revertant frequencies in the experimental groups were 8.5 (94.7% survival), 13.0 (87.8% survival), 135.2 (41.5% survival), and 411.0 (0.72% survival), respectively, for the above treatment periods per 10^6 survivors. The control frequency was $0/10^6$ survivors. The positive mutagenic effect of epichlorohydrin in *N. crassa* was also confirmed by Westergard (1957).

7.2.5 Gene Mutations in Yeast

Epichlorohydrin was found to induce gene mutations and other types of genetic damage in yeast. Vashishat et al. (1980) investigated the ability of epichlorohydrin to induce reverse gene mutation, mitotic crossing-over, and gene conversion in a diploid strain of the yeast *Saccharomyces cerevisiae* D7. Two batches of cultures were used and assayed for cross-overs, revertants, and convertants: one batch was treated with 0.065 M epichlorohydrin for 0, 5, 10, 15, and 20 minutes, and another batch of cultures was treated with 0.13 M epichlorohydrin for 0, 5, and 10 minutes. In the first batch, the cross-over frequencies were 0.13, 0.40, 0.59, 1.68, and 2.17 percent, revertants/10^7 survivors were 30, 190, 366, 427, and 297; and convertants/10^6 were 27, 59, 113, 183, and 297, respectively, for 0, 5, 10, 15, and 20 minutes of treatment. In the second batch, the cross-over frequencies were 0.15, 0.55, and 1.39 percent revertants/10^7; survivors were 46, 326, and 547; and convertants/10^6 were 33, 109, and 330, respectively, for 0, 5, and 10 minutes of treatment, indicating that epichlorohydrin was mutagenic in the yeast. Sora et al. (1979) also reported the induction of gene mutations both of base-pair substitution and insertion/deletion-type, mitotic crossing-over, and mitotic gene conversion in the yeast. However, these investigators did not provide data to support their claim. Heslot (1962) reported (abstract) the induction of Arg^+ mutations in *Schizosaccharomyces pombe* by epichlorohydrin.

7.2.6 Gene Mutations in Mammalian Cell Cultures

Moore-Brown and Clive (1979) demonstrated the induction of gene mutations at the thymidine kinase (TK) locus in mouse lymphoma cells in vitro by 0.21, 0.42, and 0.65 umoles of epichlorohydrin. Two types of mutant colonies (TK-/-), large and small, were induced by epichlorohydrin. The large mutant colonies followed a linear dose-response relationship indicating a typical one hit point mutational mechanism. However, the dose induction curve for the small colonies indicated a multihit mutational mechanism. From the shape of the dose-response curve (Figure 7-12), there appears to be little doubt about the mutagenic potential of epichlorohydrin in cultured mammalian cells.

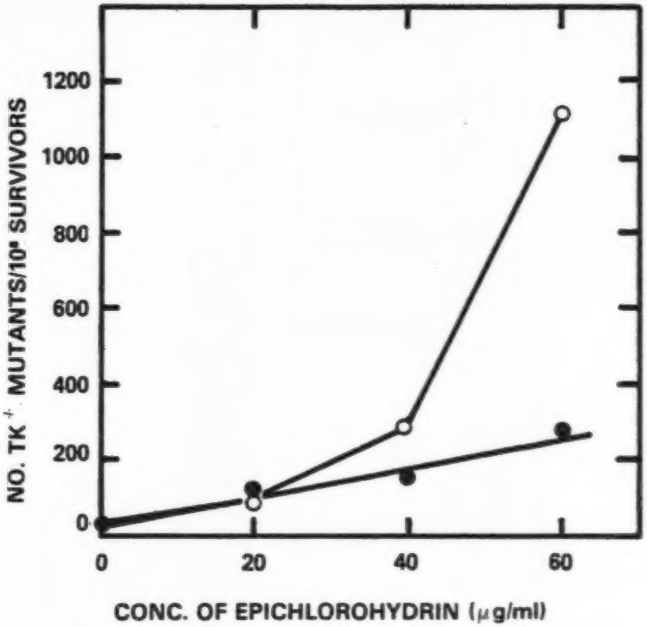

Figure 7-12. Dose-response curve for epichlorohydrin-treated cultures. The total mutant frequency is divided to show TK$^{-/-}$ mutant induction of large colony (●) and small-colony (○) mutants versus the concentration of epichlorohydrin.

7.2.7 Sex-Linked Recessive Lethal Test in Drosophila

Epichlorohydrin was tested for the induction of sex-linked recessive lethal mutations in Drosophila.

Rapoport (1948) analyzed 526 chromosomes from the experimental and 887 chromosomes from the control groups. The frequencies of sex-linked recessive lethal mutations were 0.7 percent and 0 percent, respectively, in the experimental and control groups, indicating epichlorohydrin was mutagenic in Drosophila. Details about the concentration of the test compound and experimental conditions were not given in the paper.

The observations of Knapp et al. (1982) indicated that epichlorohydrin induced sex-linked recessive lethal mutations in Drosophila (Table 7-9). Flies were exposed to epichlorohydrin by injection and feeding methods. In the injection method, 4-day-old male flies (Oregon-K) were given 2.6, 5.1, 25.5 μmoles of epichlorohydrin and individually mated to 3 Basc females per

TABLE 7-9. INDUCTION OF SEX-LINKED RECESSIVE LETHALS IN *DROSOPHILA* BY *EPICHLOROHYDRIN*

Experiment No.	Concentration (mM)	Method of Administration	Brood A		Brood B		Brood C		Brood D		Brood E	
			No. of Chromosomes	% Lethal	No. of Chromosomes	% Lethal	No. of Chromosomes	% Lethal	No. of Chromosomes	% Lethal	No. of Chromosomes	% Lethal
1	2.6	Injection	526	0	537	0.93	521	0.19	525	0	465	0
2	5.1	Injection	958	0.52	1057	0.47	957	0.31	1018	0.10	1108	0.45
3	25.5	Injection	448	0.67	528	1.33	412	0.49	450	0.22	252	0.79
4	25.5	Injection	922	1.74	836	0.96	801	0.37	844	0.47	817	0.12
5	2.6	24-h feeding	628	0	772	0	580	0	704	0.14	--	--
6	5.1	24-h feeding	773	0.13	701	0	702	0.28	603	0	811	0
C[b]	--	--	5216	0.17	5027	0.22	5247	0.17	2050	0.34	1735	0.17

C = Accumulated laboratory control.

NOTE: Four-day-old Oregon-K males were treated and individually mated to three Basc females (virgins) per brood; 0.7% NaCl or 5% sucrose was added in injection and feeding solutions, respectively. After injection of 25.5 mM, 75 percent of the flies were fertile through brood E; after feeding of 2.6 mM, this was the same; during feeding of 5.1 mM, however, 70 percent of the flies died within 24 hours, while 25.5 mM was 100 percent lethal; here a higher stickiness of the solution may have played a role. DMSO was used (except in experiment 6) as an auxiliary solvent at final concentrations of 0.5% or lower, although it was not necessary to get perfect mixing of epichlorohydrin in water.

Source: Knaap et al. (1982).

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brood. A concentration of 2.6 mM epichlorohydrin induced sex-linked recessive lethals in broods B and C but not in broods A, D, and E. However, the concentrations of 5.1 and 25.5 mM induced sex-linked recessive lethal mutations in all five broods. In the feeding method at the concentration of 2.6 mM, no sex-linked recessive lethals were found. The other two concentrations, 5.1 and 25.5 mM, were toxic to the flies, resulting in 70 percent and 100 percent mortality, respectively. This study also indicates that the negative results with the feeding study may be due to the fact that flies did not consume the test compound in sufficient quantities or that the test compound was unable to reach germ cells probably because of its rapid metabolism by the other organs in the body.

Wurgler and Graf (1981) tested epichlorohydrin in the Drosophila sex-linked recessive lethal test and found it to be negative. Flies, Berlin-K 2-day-old males, were fed with 0.2 percent of epichlorohydrin for 3 days on glass filters. Epichlorohydrin was dissolved in 2 percent DMSO and then diluted in buffered 5 percent sucrose solution (pH 6.8) before feeding. In 2,209 chromosomes tested in broods, days 1-3, there were 7 or 0.32 percent recessive lethals. In water and solvent controls, the recessive lethal frequencies were 0.28 percent and 0.40 percent, respectively. The negative response in this experiment is probably due to the problems found in feeding studies such as the flies not eating sufficient amounts of the test compound, or the rapid metabolism and distribution of the compound in other tissues so that it was unable to reach the germ cells. If injection studies were performed in this experiment, a positive response may have been obtained. The injection studies of Kramers (quoted by Vogel et al. 1981) conclusively show that epichlorohydrin (purity not given) is mutagenic in Drosophila sex-linked recessive lethal mutation tests.

7.2.8 Chromosomal Aberrations in Human and Other Mammalian Systems

7.2.8.1 Studies on Human Chromosomes in Vitro--Kucerova et al. (1976) investigated the cytogenetic effects of epichlorohydrin in cultured human blood lymphocytes. Blood samples were obtained from two healthy donors (one male and one female) and cultured for 56 hours. Two series of experiments were conducted. In the first series of experiments, epichlorohydrin

(Czechoslovak Chemical Industry) was added for the last 24 hours of cultivation at concentrations of 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9}, 10^{-10}, 10^{-11}, and 1^{-14} M. In the second series of experiments, cells were exposed to 10^{-4} and 10^{-5} M concentrations of epichlorohydrin in three ways: (1) for 1 hour before the beginning of cultivation (G_0); (2) for 1 hour between the 24th and 25th hour of cultivation (G_1); and (3) for the last 24 hours of cultivation (δ). Chromosome preparations were stained with Giemsa and 100 metaphases were scored for each dose. Aberrations were classified as chromatid breaks, chromatid exchanges, chromosome breaks, and chromosome exchanges. Gaps were scored separately. In the first series of experiments, a dose-related response of chromosomal aberrations was obtained. The aberration frequencies were 8.9, 3.3, 1.3, 1.0, 1.7, and 0.7/100 metaphases, respectively, for the above doses. The control frequency was 1 aberration/100 metaphases. Chromosome and chromatid breaks were the most common type of aberrations found. In the second series of experiments, no differences were found between the treated (G_0 and G_1) and control groups. However, in the 24-hour treatment group, the concentration of 10^{-4} M was too toxic; only 10^{-5} M increased the number of aberrations (9.2/100 metaphases) compared to the control value of 1.9 aberrations in 100 metaphases. Appropriate positive (TEPA) and solvent (DMSO) controls were employed in this study.

Kucerova and Polivkova (1976) tested the clastogenic effect of 10^{-6} M epichlorohydrin in cultured human lymphocytes 1 hour before initiation (G_0) and 24 hours after the initiation of DNA synthesis (S), using conventional and Giemsa-banding (G-banding) procedures. One hundred metaphases from conventional staining and 100 metaphases from G-banding procedures were analyzed for chromosomal aberrations. The banded preparations according to these authors exhibited higher incidence of aberrations, 6 percent at 1 and 18 percent at 28 hours of treatment, as compared to frequencies of 2.5 percent and 1.5 percent aberrations for the same periods of treatment in conventionally stained chromosome preparations. In the negative controls, there were 0.7 percent aberrations in conventionally stained, and 3 percent in banded preparations. Solvent and positive controls were also used in these studies. Even though increased aberration frequencies were noted in the banded chromosomes by these investigators, the banding technique in general has been very rarely used in screening chemicals for mutagenic activity. These investigators studied only one concentration of the chemical and consequently no dose-response data are available. Due to these shortcomings, this report cannot be evaluated critically.

Norppa et al. (1981) also demonstrated the clastogenic effects of epichlorohydrin in cultured human lymphocytes. Concentrations of 0.05, 0.20, 0.40 mM epichlorohydrin induced 0 percent, 18 percent, and 13.3 percent aberrations. In controls, the frequency of aberration was 0.5 percent. Two hundred metaphases per dose from replicate cultures were scored. Epichlorohydrin was dissolved in acetone prior to use. Fisher's exact probability test (one-tailed) was used to analyze the data.

7.2.8.2 Studies on Rodent Chromosomes in Vitro--Negative results on the clastogenic effects of epichlorohydrin in cultured rat liver cell line (RL1) were obtained by Dean and Hodson-Walker (1979). Epichlorohydrin at concentrations of 5, 10, 15, 20 µg/ml induced 0.8, 0.9, 0.8, and 1.5 percent aberrations, respectively, as compared to the controls (0.6%). The median lethal concentration (LC_{50}) was 40 µg/ml. It appears rat liver cells are resistant to the clastogenic action of epichlorohydrin through detoxification.

7.2.8.3 Studies on Human Chromosomes in Vivo--Humans occupationally exposed to epichlorohydrin have been examined for chromosomal abnormalities in their blood lymphocytes. Positive results were reported by many investigators (Kucerova et al. 1977; Sram et al. 1976; Picciano 1979).

Kucerova et al. (1977) examined 35 workers 23 to 54 years of age before they started work, 1 year after they started work, and 2 years after they started work in a newly established chemical plant manufacturing epichlorohydrin. The workers were not previously exposed to either radiation or drugs. According to these investigators, the concentration of epichlorohydrin to which the workers were exposed exceeded the limits (1 mg/m^3) of acceptable concentration in Czechoslovakia. Chromosome preparations were made from blood samples cultured for 56-58 hours and stained with Giemsa. Slides were coded and scored blind by two collaborating laboratories. Two hundred metaphases were scored from each worker for each of three intervals. Data were analyzed with the X^2 test. Before the workers started to work in the epichlorohydrin manufacturing plant, they had an average frequency of 1.37 percent ± aberrations. One year after they started to work, the aberration frequency increased to 1.91 percent; 2 years later the aberration frequency increased to 2.69%. Statistical analysis revealed that the aberration frequency in workers exposed for 2 years was highly significant ($P<0.0001$) as compared to controls. The aberrations were mostly in the form of chromatid and chromosomal breaks.

The results of Kucerova et al. (1977) were confirmed by Picciano (1979) in the United States. Picciano (1979) examined the blood lymphocytes of 93 workers (20-62 years of age) exposed to epichlorohydrin and 75 matching controls (20-49 years of age). Two hundred cells from each individual were analyzed by five independent laboratories. Picciano indicated that the details of exposure data were not available to him. The aberration data were analyzed using the X^2 test. In the exposed workers, there were 4.34 percent chromatid breaks, 0.96 percent chromosome breaks, 0.13 percent marker chromosomes, 0.12 percent severely damaged cells, and 4.25 percent abnormal cells with a total of 9.80 percent aberrations. In the controls there were 2.15 percent chromatid breaks, 0.51 percent chromosome breaks, 0.08 percent marker chromosomes, 0.01 percent severely damaged cells, and 2.38 percent abnormal cells with a total of 5.13 percent aberrations.

Sram et al. (1980) examined 28 workers, 34 matching controls, and 21 general population subjects. None of the subjects was previously exposed to radiation or other mutagenic chemicals according to these investigators. Epichlorohydrin concentration in the exposed workers ranged over the maximally permitted concentration limits (1 mg/m^3) in the last 2 years prior to chromosome analysis. The following frequencies of aberrant cells bearing chromosome and chromatid breaks were detected in the various groups. In the epichlorohydrin exposed group, the aberration frequency was 3.12 percent, in matching controls the aberration frequency was 2.06 percent, and in the general population control group the frequency was 1.33 percent. Statistical analysis revealed significant difference between the epichlorohydrin group and the matching control group ($P<0.05$). Similarly, significant differences were found between the epichlorohydrin exposed group and the general population control group ($P<0.01$).

7.2.8.4 Studies on Rodent Chromosomes in Vivo--Clastogenic effects of epichlorohydrin in vivo have been reported in the bone marrow cells of laboratory rodents (Sram et al. 1976, 1981; Dabney et al. 1979).

Sram et al. (1976) studied the in vivo clastogenic effects of epichlorohydrin in mouse bone marrow cells. Epichlorohydrin (LD_{50} was 100 mg/kg) was administered by both intraperitoneal and oral routes. Chromosome analysis from bone marrow cells of mice injected intraperitoneally with 1, 3, 5, 10, 20, and 50 mg/kg for 24 hours revealed 2.8, 6.0, 10.0, 27.2, and 20.4 percent aberrant cells, respectively, with a clear dose-response relationship. In DMSO control animals, 4.0 percent of the bone marrow cells exhibited chromosomal aberrations. High frequencies of aberrant cells were also noted in mice injected with subacute doses (five daily injections of 5, 10, and 20 mg/kg of epichlorohydrin). The incidence of cells with chromosome aberrations were 38.0, 36.0, and 80.4 percent, respectively, for these doses. When epichlorohydrin was given orally at 5, 20, 40, and 100 mg/kg, dose-related increases in the incidence of aberrations (6, 24.0, 22.4, and 29.5 percent, respectively) were noted; these were mainly in the form of chromatid breaks.

Sram et al. (1981), in a review article, refers to a paper by these same authors (1976) in which they reportedly investigated the clastogenic effect of epichlorohydrin in the bone marrow cells of the Chinese hamster. The incidence of aberrations at 5-20 mg/kg of epichlorohydrin was 2.0-2.4 percent compared to the control frequency of 0.6 percent aberrations. However, Sram et al. (1976) does not reveal such a report of chromosomal studies in the Chinese hamster.

Dabney et al. (1979), as cited by Sram et al. (1981), failed to detect chromosomal aberrations in groups of 10 male and 10 female rats exposed to epichlorohydrin at 0, 5, 25, or 50 ppm for 6 hours/day, days/week for 4 weeks in air. The aberration frequencies in treated groups were 0.1-0.4 percent. It appears that rat bone marrow cells are relatively insensitive to epichlorohydrin compared to mice. See also earlier reference to the rat liver cell line (RL1).

In vivo chromosomal aberration studies indicate that epichlorohydrin is mutagenic in mouse bone marrow cells but not in rat bone marrow cells. The difference is probably due to the fact that rats are resistant to the effects of epichlorohydrin. Such an observation was also made by Norppa et al. (1981) in cultured rat liver cells.

7.2.8.5 Micronucleus Assay--Epichlorohydrin was tested for its ability to induce micronuclei in the bone marrow cells of mice (Kirkhart 1981; Tsuchimoto and Matter 1981). Micronuclei are formed when chromosome fragments that lack centromeres fail to incorporate into daughter nuclei and these can be detected in polychromatic erythrocytes (PCEs) of the bone marrow under the microscope.

Kirkhart (1981) demonstrated the negative response of epichlorohydrin in mice. Mice were treated i.p. twice, once at 0 and the other at 24 hours, with the test compound at concentrations of 0.0225 mg/kg (12.5% LD_{50}), 0.045 mg/kg (25% LD_{50}), and 0.09 mg/kg (50% LD_{50}). They were sacrificed 6 hours after the second injection. Bone marrow smears were made for each concentration and 1,000 polychromatic erythrocytes (PCEs) were examined for the presence of micronuclei. The frequencies of micronuclei were 10, 15, and 12 per 1,000 PCEs, respectively, for the above doses. In the negative control, the frequency of micronuclei was 4 per 1,000 PCEs and in the positive (TMP) controls the frequency was 113 per 1,000 PCEs. Statistical analysis (Mackey and MacGregor 1979) revealed no significant differences between negative controls and experimental groups.

Tsuchimoto and Matter (1981) also reported the negative response of epichlorohydrin in the micronucleus assay. At concentrations of 0.02 (12-5% of LD_{50}), 0.04 (25% of LD_{50}), and 0.08 (50% of LD_{50}) mg/kg, the test compound induced 0.10, 0.08, and 0.10 percent micronuclei as compared to 0.05% micronuclei in the negative control. The criteria set for positive conclusion were: (1) two or more mice per group with micronucleated polychromatic erythrocyte frequencies above 0.40 percent, (2) one or more treated groups with mean polychromatic erythrocytes frequencies above 0.30 percent, and (3) statistical significance (Kastenbaum and Bowman 1970) in one or more treated groups. Epichlorohydrin did not meet any of these criteria and thus concluded as negative by these investigators.

It should be noted that the failure of epichlorohydrin to induce micronuclei does not necessarily mean that the test compound is not mutagenic. It may be that chromosome aberrations that were induced were probably of reciprocal exchange type and consequently no micronuclei were formed.

7.2.8.6 Dominant Lethal Assay--The dominant lethal assay detects dominant lethal effects induced by chemical mutagens in parental germ cells. The germ cells carrying dominant mutations when they fertilize normal counterparts result in the death of the fetuses during development, which can be scored and evaluated. The dominant lethal assay generally involves treatment of the male parent with single or multiple doses of the mutagen and breeding the treated males with virgin females for eight weeks. Mated females are sacrificed at mid-pregnancy and uterine analysis is made for total implants, live implants, and dead implants, and compared with controls to determine the incidence of dominant lethals.

Epstein et al. (1972), in a survey of 174 chemicals, tested epichlorohydrin for the induction of dominant lethal effects in mice. Male mice (group of 10) were treated intraperitoneally with 150 mg/kg of epichlorohydrin (purity not given) and bred with untreated females for 8 consecutive weeks (number of female mice per week not given). The dominant lethal analysis revealed no differences in total implants and fetal deaths between the experimental and control groups. However, details were not provided in this report.

Sram et al. (1976) also reported negative results with epichlorohydrin in the dominant lethal assay in mice. The test compound at concentrations of 5, 10, and 20 mg/kg was injected intraperitoneally and at concentrations of 20 and 40 mg/kg administered orally with acute (single dose) and subacute (1 dose/day for 5 days) doses. No differences in the frequency of dominant lethal mutations were noted between the experimental and control groups.

It should be noted that the dominant lethal assay may not be sensitive to epichlorohydrin. The negative results in this assay may not necessarily mean that the test compound is not mutagenic; it is possible that epichlorohydrin is unable to reach mammalian germ cells in sufficient quantity to cause dominant lethal effects or it may not reach the germ cells at all. However, more information is needed before reaching such a conclusion that epichlorohydrin is not a germ cell mutagen.

7.2.8.7 Sister-Chromatid Exchange Assay--The sister-chromatid exchange (SCE) assay detects reciprocal exchanges induced by mutagenic agents between sister chromatids of chromosomes. Epichlorohydrin was found to induce SCEs in cultured human lymphocytes (White 1980; Carbone et al. 1981; Norppa et al. 1981).

White (1980) studied the effects of epichlorohydrin on the frequencies of sister-chromatid exchanges in the lymphocytes of two female healthy adult donors. The lymphocyte cultures were exposed to epichlorohydrin as follows: (1) cultures were exposed to 1×10^{-3}, 4×10^{-4}, 2×10^{-4}, 1 x

10^{-4}, 8×10^{-4}, and 4×10^{-5} M concentrations of epichlorohydrin for the entire culture period of 73 hours, (2) cultures were exposed for the final 25 hours of cultivation with above concentrations of epichlorohydrin, and (3) cultures were exposed for 2 hours (48-50 hours of cultivation) with 1×10^{-3}, 4×10^{-4}, 2×10^{-4}, and 1×10^{-4} M concentrations of epichlorohydrin. Chromosome preparations were stained with the fluorescence plus Giemsa (FPG) technique of Perry and Wolff (1974) to differentiate sister chromatids. Twenty to 30 metaphases were scored and the frequency was expressed as SCE/cell. In cultures exposed for 73 hours, there were 14.8 ± 0.73, 12.6 ± 0.79, 10.1 ± 0.53 SCEs/cell indicating a dose-related response. The other three concentrations, 1×10^{-3}, 8×10^{-5}, 4×10^{-4}, and 2×10^{-4} M, yielded no mitoses. The control frequency was 8.2 ± 0.53 SCE/mM. Similar dose-related increases in SCEs were also noted for cultures treated for 25 hours of cultivation was threefold higher (19.5 ± 1.01/cell) at the concentration of 4×10^{-4} M, as compared to control frequency (6.6 ± 0.49/cell) in the absence of metabolic activation.

Carbone et al. (1981) demonstrated the induction of SCEs in cultured human blood lymphcytes with low concentrations of 1×10^{-5}, 1×10^{-8}, and 1×10^{-11} M of epichlorohydrin in the absence of metabolic activation. The frequencies of SCEs were analyzed with the Student's t-test, and the results were significant at concentrations of 1×10^{-5} M ($p<0.001$) and 1×10^{-8} M ($p<0.05$) compared to controls.

Norppa et al. (1981) also demonstrated the induction of SCEs in human blood lymphocytes. Epichlorohydrin at concentrations of 0.05, 0.20, and 0.40 mM induced 8.4 ± 0.4, 30.5 ± 1.2, and 56.3 ± 2.8 SCEs/cell, respectively. The solvent control frequency was 7.0 ± 0.3/cell. There is a clear dose-response relationship between the number of SCEs and the concentrations of epichlorohydrin. The data were analyzed using the Student's t-test and found that the experimental groups exhibited statistical significance over the control value.

7.2.9 Conclusions

Epichlorohydrin has been demonstrated to be mutagenic in both prokaryotic and eukaryotic systems. This compound has been shown to be an active inducer of gene mutations in bacteria, Neurospora, yeast, cultured mammalian cells, and Drosophila. Epichlorohydrin was also effective in causing sister-chromatid exchanges in human cells in vitro and preferential cell killing of repair-deficient bacteria. Chromosomal effects induced by epichlorohydrin were detected both in vivo and in vitro mammalian assays. The micronucleus assay, however, indicated a negative response of epichlorohydrin presumably because the aberrations induced were reciprocal exchanges that segregated without forming micronuclei. The dominant lethal assay in mice also produced negative results. However, the assay may not be sensitive enough to detect mutations other than gross chromosomal aberrations. Based on the above weight-of-evidence, epichlorohydrin should be regarded as mutagenic, thus having the potential to cause somatic mutations, which may be involved in the etiology of cancer in humans. The concern is also raised that epichlorohydrin may reach germ cells; however, additional studies are required before concluding that epichlorohydrin is not a germ cell mutagen in mammals.

7.3 REPRODUCTIVE AND TERATOGENIC EFFECTS

7.3.1 Reproductive Effects

A qualitative assessment of the available data was conducted to determine whether epichlorohydrin has the potential to cause adverse reproductive or developmental effects. Six studies have been reviewed concerning the effect of epichlorohydrin on the reproductive ability of male and female rats and rabbits; on the development of offspring in the rat, mouse, and rabbit; and on the semen of workers exposed to epichlorohydrin. Hahn (1970) was the first to investigate antifertility effects in male rats due to epichlorohydrin. Male Sprague-Dawley rats (quantity not stated) were administered 15 mg/kg epichlorohydrin orally for 12 days. There was no observed histologic change in the testes, epididymis, prostate, or seminal vesicles after 12 days of exposure, nor was sexual libido or ejaculatory ability affected. However, temporary sterility was produced in the males. After 1 week of exposure, the male rats were unable to impregnate proestrous female rats, with the effect reversed 1 week after discontinuation of treatment.

Cooper et al. (1974) studied the effects of epichlorohydrin and several related compounds. Adult Wistar rats (five per group) were given epichlorohydrin orally in suspensions of arachis oil at doses of 20-100 mg/kg and then were sequentially mated to unexposed females for 10 consecutive weeks. When given at 50 mg/kg/day for 5 consecutive days, epichlorohydrin rendered male rats totally incapable of impregnating unexposed female rats. When animals were exposed to only a single dose of epichlorohydrin at 100 mg/kg, fertility was reduced but not completely abolished (see Table 7-10). With a single dose of 100 mg/kg epichlorhydrin, no histologic effects were observed after 8 weeks. However, after 12 weeks, lesions were observed in the efferent ductus, and large retention cysts were present in the ductuli efferentes and proximal caput in 4 of 5 animals.

TABLE 7-10. THE EFFECTS OF EPICHLOROHYDRIN ON THE FERTILITY OF WISTAR RATS

Compound	No. of Days of Exposure	Daily Dose (mg/kg)	Weeks:	Average Weekly Litter Size 1	2	3	4	5	6	7	8	9	10
alpha-Chlorohydrin	5	20		0	0	7	9	3					
	1	10		0	0	0	0	0	0	0	0	0	0
Epichlorohydrin	5	20		0	0	0	11	11					
	5	50		0	0	0	0	0	0	0	0	0	0
	1	100		0	4	3	4	4	2	2	4	2	3

Five Wistar rats used for each dose level.

Source: Cooper et al. (1974)

The Toxicology Research Laboratory, Dow Chemical Company (John et al. 1979) conducted a three-part study evaluating the reproductive ability of the male rabbit, the male rat, and female rat after exposure to epichlorohydrin. Groups of 10 male rabbits (New Zealand), 30 male rats (Sprague-Dawley), and 30 female rats (Sprague-Dawley) were exposed for 10 weeks by inhalation (6 hours/day, 5 days/week) to 0, 5, 25, or 50 ppm production grade epichlorohydrin supplied by Dow Chemical Company (analyzed as 98.8% pure by weight with 0.03% propylene dichloride, 0.08% cis-1,3-dichloropropane, 0.07% 2,3-dichloropropene, and 0.01% beta-chloroalkyl alcohol). The quality and quantity of rabbit semen was evaluated every week for 2 weeks prior to exposure, every week for 10 weeks during exposure, and every other week for 10 weeks after exposure (see Table 7-11). After 10 weeks of exposure, the male rabbits were mated to untreated female rabbits in estrus; the females

TABLE 7-11. THE EFFECTS OF INHALED EPICHLOROHYDRIN ON THE SEMEN OF RABBITS AND ON THE FERTILITY OF MALE AND FEMALE RATS[a]

Compound:	Epichlorohydrin																							
Species:	New Zealand white rabbits (males) and Sprague-Dawley rats (males and females)																							
Exposure:	Inhalation, 6 hours/day, 5 days/week, 20 weeks																							
Level:	0, 5, 25, and 50 ppm																							
Group Sizes:	10 male rabbits, 30 male and 30 female rats per level of exposure																							
		PREEXPOSURE								EXPOSURE									POSTEXPOSURE					
RABBITS	Weeks:	-2	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Semen evaluation		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X		X		X		X
Mating														X										
RATS																								
Fertility:	Males (matings)			X		X		X			X			X		X								X
	Females (matings)													x										

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[a] Toxicology Research Laboratory, Dow Chemical Company.

Source: John et al. (1979).

were subsequently induced to ovulate with human chorionic gonadotrophin. On day 28 of gestation, the female rabbits were sacrificed, and the number of corpora lutea, implantation sites, and resorbed fetuses were recorded.

To evaluate fertility in male rats, males were exposed to epichlorohydrin for 10 weeks; then the exposed male rats were mated to two unexposed female rats for 1 week of cohabitation initiated on the 2nd, 4th, 7th, 10th, 12th, and 20th week of study. The untreated female rats were sacrificed 12 days after the last day of cohabitation, and examined for the number of corpora lutea, implantation sites, and resorption sites. To evaluate fertility in female rats the animals were exposed for 10 weeks and then were allowed to mate with two different unexposed male rats for 2 consecutive 5-day periods. The stage of estrus was evaluated in daily vaginal smears until sperm was observed in the vagina. The date of delivery, the number of live and dead pups, and observations of external abnormalities were recorded at birth.

In this study (John et al. 1979), the body weights, clinical chemistry, number of corpora lutea, and semen parameters were evaluated statistically by one-way analysis of variance and Dunnett's test. Preimplantation loss and numbers of resorptions were analyzed by the Wilcoxan test modified by Haseman and Hoel. The fertility index was analyzed by Fisher's exact probability test.

In this study, exposure to epichlorohydrin produced signs of toxicity in rats. Male and female rats exposed to 50 ppm epichlorohydrin but not 5 or 25 ppm, gained significantly less weight during the 10-week exposure period than the controls. Male and female rats exposed to 25 ppm had slight increases in both absolute and relative kidney weights, whereas those exposed to 50 ppm had significant increases. The livers of males at all exposure levels and the livers of females exposed to 50 ppm were slightly but not statistically heavier than the controls. In addition, histopathologic changes were observed in the nasal turbinates of male and female rats exposed to 25 and 50 ppm epichlorohydrin. It is possible that both control and experimental rats were ill prior to treatment, since white blood cell counts were elevated during the preexposure period with symptoms of sialodacryoadenitis observed during the first 2 weeks of exposure in all groups.

In male rats, 25 and 50 ppm epichlorohydrin markedly affected the ability of the animals to impregnate unexposed female rats. After the females were mated with the exposed males, there were significantly fewer implantation sites in female rats mated to males exposed to 25 and 50 ppm, but not 5 ppm epichlorohydrin. The matings conducted during weeks 12-20 of the experiment (2-10 weeks after discontinuation of exposure) did not result in significant reduction in implantation sites, suggesting that this effect was reversible. There was an increase in preimplantation loss in females mated to males exposed to 25 and 50 ppm epichlorohydrin. This preimplantation loss was observed in matings (at 25 and 50 ppm) conducted during the exposure period, while preimplantation losses in matings conducted during the recovery period were observed only in groups exposed to 50 ppm. A reduction in the number of corpora lutea was observed in females mated with males (i.e., those exposed to 50 ppm epichlorohydrin) during weeks 2, 4, 7, and 10 but not weeks 12-20.

In studies on the female rat (John et al. 1979), exposure to 5, 25, or 50 ppm epichlorhydrin did not affect the animal's ability to become pregnant, the length of gestation, litter size, survival indices, sex ratios, or the incidence of malformations in the offspring.

In the study using male rabbits, signs of toxicity were observed in animals exposed to 50 ppm epichlorohydrin (John et al. 1979). Male rabbits at this dose level gained significantly less weight than controls during the 10-week exposure period, with two rabbits dying spontaneously or sacrificed due to moribund conditions. Epichlorohydrin exposure apparently did not alter semen volume, sperm concentrations, motility, or morphology in rabbits. During the 10th week of the experiment, each male was mated to unexposed females. There were no dose-related alterations in fertility, implantations, corpora lutea, or resorptions in the unexposed female rabbits.

7.3.1.1 Male Clinical-Epidemiologic Investigations--Milby et al. (1981) conducted a clinical-epidemiologic investigation of testicular function in chemical plant workers occupationally exposed to epichlorohydrin. Men working on the Shell Chemical Corporation plants in Deer Park, Texas (plant A, epichlorohydrin production since 1948), and Norco, Louisiana (plant B, production since 1955), was included in this study. Semen samples were obtained as well as blood samples for measurement of follicle stimulating hormone (FSH) and luteinizing hormone (LH). There was some attempt at evaluating the intensity of exposure (exposure estimates from industrial hygiene survey, personal exposure knowledge, plant employment records) from the men in plants A and B.

It was not possible to evaluate unexposed workers for use as controls from either of the two plants. The control population was selected from chemical plant workers previously evaluated by the authors in other studies. The men used as controls had no history of exposure to testicular toxicants (90 men were used as controls).

The results of this study indicated that the frequency distribution of sperm in the semen collected from 44 men in plant A and 87 men in plant B did not significantly differ from that of the control populations. There was no association suggestive of deleterious effect with either duration or intensity of exposure. In addition, there were no significant differences in hormone concentrations (FSH, LH, testosterone). However, these results do not conclusively determine whether epichlorohydrin produces adverse testicular effects in humans. It is recognized that this type of study has major inherent weaknesses due to confounding factors related to the participation of all men potentially exposed to epichlorohydrin. In addition, data such as marital status of the men, age, and number of children were not available to the authors. Therefore, it was difficult to assess whether the population studied was a true representative population of all male workers exposed to epichlorohydrin or whether the control population represented a true distribution of all fertile men. The most critical weakness with this study was that there were no actual exposure measurements; the exposure intensity and duration was estimated by job category or by a combination of judgments based upon industrial hygiene sampling and the investigator's appraisal of the work situation.

7.3.2. Teratogenic Effects

The teratogenic potential of epichlorohydrin has been evaluated in two studies (Pilny et al. 1979; Marks et al. 1982). Pilney et al. (1979) evaluated a small number of rats and rabbits to establish a dose for maternal toxic effects (tolerance study) and then conducted a teratology study using larger numbers of rats (Sprague-Dawley) and rabbits (New Zealand). For the tolerance study, five or six pregnant rats and five pregnant rabbits were exposed to 0, 25, 50, or 100 ppm epichlorohydrin (containing by weight 99.8 percent epichlorohydrin, 0.11 percent 2,3-dichloropropene, 0.03 percent cis-1,3-dichloropropene, and 0.01 percent beta-chloroallyl alcohol) administered by inhalation and analyzed by the Dow Chemical Company, Freeport, Texas. Concurrent controls were exposed to filtered air. Exposure to 50 and 100 ppm epichlorohydrin in the tolerance study produced signs of maternal toxicity (decrease in maternal weight gain and decrease in intra-abdominal adipose tissue). In the groups exposed to 100 ppm epichlorohydrin, three of the six animals had only resorption sites, two animals had no implantation sites, and the one remaining had normal appearing fetuses. The rabbits exposed to 50 and 100 ppm epichlorohydrin had signs of maternal toxicity (decrease in maternal weight gains, increased respiratory tract infections), but no fetal loss. Based upon the results of the tolerance study, the teratology study was conducted with doses of 2.5 and 25 ppm epichlorohydrin to avoid problems with severe maternal toxicity. The teratology study utilized 43-66 rats and 20-25 rabbits. The rats and rabbits were exposed for 7 hours/day on days 6-15 or 6-18 of gestation, respectively.

The data in this study (Pilney et al. 1979) were analyzed statistically using the Wilcoxan test modified by Haseman and Hoel for evaluating frequency of resorption among litters and fetuses. Analysis of percent pregnant, maternal survival rate, and other incidence data were made by Fisher's exact probability test. Analyses of fetal body weight, body length, maternal weight gain, and maternal organ weights were made by analysis of variance. Group means were compared to control values using Dunnett's test. The level of significance was chosen at $p<0.05$.

Pilney et al. (1979) reported signs of maternal toxicity in rats exposed to 25 ppm epichlorohydrin, but not to 2.5 ppm epichlorohydrin. Rats exposed to 25 ppm weighed less (statistically significant) than the control animals throughout the exposure period, and consumed significantly more water. There were no signs of maternal toxicity observed in rabbits. There were no alterations in pregnancy rates, number of litters, corpora lutea, implantation sites, resorption site, numbers of dead fetuses, fetal body weight or crown-rump length, or incidence of malformation in either rats or rabbits.

Marks et al. (1982) evaluated the teratogenic potential of epichlorohydrin administered to rats and mice. Epichlorohydrin (laboratory grade Fisher Scientific Co.) was administered by gastric intubation in doses of 0, 40, 80, and 160 mg/kg/day to 14-35 outbred albino rats (CD, 176-200 g). Epichlorohydrin was administered by gastric intubation in doses of 80, 120, and 160 mg/kg/day to 25-49 outbred albino mice (CD-1, 60-90 days old). The chemical was dissolved in cottonseed oil and administered to both rats and mice on days 6-15 of gestation.

In this study (Marks et al. 1982), the data were analyzed statistically to evaluate differences between the groups using the Mann-Whitney U-test or Student's t-test. Differences in the dose-response relationship were evaluated using Jonckheere's test. Two-tailed analysis was performed and the level of significance was chosen at $p<0.05$.

In this study (Marks et al. 1982), both the rats and mice showed signs of maternal toxicity at the two highest doses administered. Rats administered 160 mg/kg/day had significantly greater increases in liver weight and 3 of 27 rats died; and at 80 mg/kg/day the epichlorohydrin caused a significant reduction in the average weight gain during pregnancy. In mice administered 160 mg/kg/day, 3 of 32 mice died, and there was a significant decrease in fetal weights. In addition, there was a significant increase in the average maternal liver weight. There were no dose-related increases in soft tissue or skeletal malformations.

7.3.3 Summary and Conclusions

Epichlorohydrin has been evaluated in six studies for its potential for causing (1) adverse reproductive effects in female rats, (2) adverse reproductive effects in male rats and rabbits, (3) adverse spermatogenic effects in humans occupationally exposed to epichlorohydrin, and (4) adverse developmental effects in rat, mouse, and rabbit concepti.

In females, epichlorohydrin has not been adequately investigated to determine if there is a potential for reproductive hazard. Only one study (John et al. 1979) has investigated reproductive effects in female rats. Animals were exposed for 10 weeks and the possible effect on future generations was not investigated. No adverse reproductive effects were observed in this study (no alteration in pregnancy rate, gestation length, litter size, survival indices, sex ratio, or external alterations). However, additional studies in the future should be conducted to firmly establish that there is no potential for harmful effects.

The data on males indicate that epichlorohydrin possesses the ability to alter male fertility. Three investigations using rats demonstrate that epichlorohydrin can cause sterility (Hahn 1970, Cooper et al. 1974, John et al. 1979). In most cases this effect is reversible (Hahn 1970; John 1979);

but with longer durations of exposure or higher concentrations, this effect may be irreversible (Cooper et al. 1974). The effect on fertility was observed with (John et al. 1979) and without (Hahn 1970; Cooper et al. 1974) other signs of toxicity (i.e., losses in the animal's body weight).

In males occupationally exposed to epichlorohydrin no alterations in sperm concentration were observed when the frequency distribution of sperm of the exposed population was compared with that of a control population (Milby et al. 1981). In addition there was no association between intensity or duration (LH, FSH, testosterone). However, since there was no concurrent control, information on reproductive history of men, or measurement of actual exposures, the full potential for adverse reproductive effects in humans cannot be adequately assessed from this study. The sensitivity of this type of clinical-epidemiologic study in detecting potential reproductive toxins has not yet been determined. Although this type of study has been used to establish a correlation between dibromochloropropane (DBCP) exposure and male sterility, it should be noted that the success of this type of study in establishing an association was dependent upon the severity of the effect. In the case of DBCP, the effects on male fertility were quite severe, with some men unable to produce sperm (azospermia) 4 years after the discontinuation of exposure.

Epichlorohyrin has been investigated for its potential to alter the development of the conceptus in rats, mice, and rabbits in two studies (Pilney et al. 1979, Marks et al. 1982). No malformations were produced even at maternally toxic doses. A reduction in fetal weights in mice were reported; however, this was observed only at doses that caused increases in liver weights in the dams (Marks et al. 1982).

In conclusion, the data available to date indicate that epichlorohydrin has the potential to produce adverse reproductive effects in the male, but not in the developing conceptus. Epichlorohydrin's ability to affect adversely male reproduction might be expected since its metabolite, alpha-chlorohydrin, is known for its antifertility properties. Alpha-chlorohydrin is thought to be produced from epichlorohydrin by the action of epoxide hydratase (Jones and O'Brien 1980) (see also section on metabolism). The antifertility effects of alpha-chlorohydrin have been studied extensively, and alpha-chlorohydrin has been shown to cause adverse reproductive effects in a number of animal species (small laboratory rodents, large domestic animals, and nonhuman primates) (Gomes 1977). Both epichlorohydrin and alpha-chlorohydrin produce the same urinary metabolites in rats (Jones et al. 1969); however, alpha-chlorohydrin appears to be more potent than epichlorohydrin in producing adverse male reproductive effects.

8. SYNERGISM AND ANTAGONISM AT THE PHYSIOLOGICAL LEVEL

No studies on the synergistic or antagonistic effects of epichlorohydrin in combination with other chemicals or conditions in humans were found in the available literature. There are few animal studies on synergistic or antagonistic effects and the studies examined are limited in scope.

Lukaneva and Rodionov (1978) studied the combined effects of epichlorohydrin and cholesterol on the development of atherosclerosis in rabbits. Five groups of rabbits (experimental details not provided) were used. The first two groups were administered epichlorohydrin orally at concentrations of 3.44 mg/kg and 17.2 mg/kg, daily for 7 months and 3.5 months, respectively. The second two groups received these two doses of epichlorohydrin plus 200 mg/kg of cholesterol according to the same schedule. The fifth group received 200 mg/kg of cholesterol for 3.5 months. Electrocardiographic monitoring of the treated animals was performed; however, no information was provided as to which animals were monitored. Serum lipid levels were assayed at 3.5 and 7 months. Rabbits were sacrificed at 3.5 and 7 months (number of animals in each sacrifice not provided), and the hearts were examined grossly and microscopically for changes.

The authors stated that the animals administered epichlorohydrin alone at doses of 17.2 mg/kg for 3.5 months had "only a few individual" electrocardiographic changes. These were not described. However, epichlorohydrin administered alone at doses of 3.44 mg/kg for 7 months led to increased atrioventricular conductivity and evidence of metabolic and functional changes in the myocardium including myocardial hypoxia.

Combined administration of epichlorohydrin at either dose and cholesterol led to a number of electrocardiographic changes characteristic of stage I atrioventricular block, and other disorders such as abnormal conductivity of the right atrium (deformation of the P wave), and increased electrical potential of the left atrium. These changes were more evident as exposure continued for longer periods (i.e., 7 months).

Epichlorohydrin administration led to an increase in the concentration of free cholesterol in the blood and hyperlipidemia (excess lipids in the blood). The administration of both epichlorohydrin at 17.2 mg/kg and cholesterol for 3.5 months led to higher concentrations of phospholipids and esterified cholesterol than did both epichlorohydrin at 3.44 mg/kg and cholesterol for 7 months; however, levels of free cholesterol and free fatty acids were higher in the animals treated with epichlorohydrin at 3.44 mg/kg for 7 months.

Shumskaya et al. (1971) examined the interaction between inhalation exposure to epichlorohydrin and subsequent exposure to cold. Three groups of 60 albino male rats (weighing approximately 180 g) were exposed to epichlorohydrin for a single exposure at concentrations of 0.007, 0.02, and 0.35 mg/l (1.85, 5.28, and 92.5 ppm) for 4 hours. A fourth group was not exposed and served as a control. After exposure, half of the animals in each group were placed in a cold chamber at 5°C for 2 hours. Several parameters were examined immediately after exposure and then within 24 hours after exposure. These included body temperature, oxygen demand, bromsulphalein retention, and levels of blood urea nitrogen, serum sulfhydryl, and liver glycogen. In addition, urinalysis was done. No description of the clinical methods of analysis was provided by the authors. Terminal organ weights were recorded 24 hours after exposure. Those animals exposed to cold stress showed fewer deviations from normal values than animals not exposed to cold stress. Body temperature decreased in rats immediately after exposure to epichlorohydrin and exposure both to epichlorohydrin and cold. After 24 hours, body temperatures were normal. Oxygen demand decreased in both cold-stressed and noncold-stressed animals. For some measurements such as bromsulphalein retention, the increases were larger in the cold-stressed rats. Urine volumes and chlorides increased and specific gravity decreased in the noncold-stressed animals only. No significant changes were observed in blood urea nitrogen, serum sulfhydryl, and liver glycogen levels in either cold-stressed or noncold-stressed animals. The parameters that showed significant variations are shown in Table 8-1.

TABLE 8-1. SUMMARY OF STUDY MEASUREMENTS AFTER EXPOSURE TO EPICHLOROHYDRIN AND SUBSEQUENT EXPOSURE TO COLD[a]

Measurement	Concentration (ppm)			
	Control	1.84	5.28	92.5
	Ambient Cold			
Body temperature, °C				
After exposure	36.6/36.7	36.2/36.3	35.5/36.1	33.4/33.5
At 24 h[b]	36.9/36.9	37.1/37.2	36.7/36.9	36.8/36.6
Oxygen demand, ml/h				
After exposure	426/414	331/348	275/264	235/251
At 24 h[b]	410/398	318/350	281/278	244/247
Kidney weight, g				
At 24 h[b]	0.83/0.86	0.87/0.83	0.87/0.84	1.02/1.01
Bromosulphalein Retention after exposure[c]	0.1/6.5	1.4/4.2	2.5/3.5	8.6/18.9
Urine				
24-h volume, ml	2.6/4.3	4.9/2.6	4.0/3.0	4.4/4.6
Total protein, g/100 ml				
After exposure[c]	7.08/6.81	6.78/7.10	6.90/7.44	7.80/8.01

[a] Source: Shumskaya et al. (1971).

[b] One day after exposure.

[c] After completion of dosage or cold exposure.

Grigorowa et al. (1977) investigated the effects of repeated epichlorohydrin inhalation exposure followed by repeated exposure to elevated ambient temperatures. Four groups of 30 male albino rats (strain and age unspecified) that weighed 220-260 g each were treated as follows:

Group 1	30 mg/m^3 epichlorohydrin--4 hours/day at 20°C
Group 2	30 mg/m^3 epichlorohydrin--4 hours/day at 20°C followed by 2 hours/day exposure to heat stress at 35°C and 50 percent relative humidity.
Group 3	no exposure to epichlorohydrin, placed in chamber--4 hours/day at 20°C
Group 4	no exposure to epichlorohydrin, placed in chamber--4 hours/day at 20°C followed by 2 hours/day exposure to heat stress at 35°C and 50 percent relative humidity.

The experimental animals were exposed daily for a 4-week period. Following exposure, the animals were maintained for an additional 2 weeks for observation. After the first day of exposure, 10 rats from each group were killed on days 10, 30, and 45, and the tissues and organs were necropsied and examined for abnormalities. The liver, kidneys, lungs, adrenals, testes, and thymus were sectioned and examined for microscopic changes. Urine was examined for aminopeptidase, albumin, volumes eliminated, excretion of phenol red, concentrations of phenol red, and sodium and potassium levels. Catalase activities in whole blood and in kidney hemogenates were determined. Aminopeptidase, glutamic-pyruvic transaminase and glutamic-oxaloacetic transaminase activities were measured in serum. Frozen sections of tissue were also examined for peroxidase, succinate dehydrogenase, and alpha-naphthylacetate activities.

In the group exposed only to epichlorohydrin (group 1), there was a slight increase in catalase activity in blood, a decrease both in the concentration and excretion of albumin in the urine, and a marked decrease in the production of urine 10 days following the initial exposure. Urine production returned to relatively normal levels in all groups 30 and 45 days following the initial treatment. Significant decreases ($p < 0.01$) in phenol red concentrations were observed in the animals exposed to both epichlorohydrin and heat stress (group 2). The phenol red concentrations for the other dosed groups were comparable to those for the controls. There was also a decrease in the rate of phenol red elimination in the urine of group 2 animals, 30 days following the initial exposure. Histopathologic examinations of the lungs, kidneys, and liver showed no significant differences between heat-stressed and nonheat-stressed animals. The animals exposed to epichlorohydrin (groups 1 and 2) showed renal toxicity including edema, degeneration of the tubular epithelium, and glomerular changes. These changes were more evident after 4 weeks of exposure. The authors concluded that heat enhanced the toxicity of epichlorohydrin.

In an additional study, Grigorowa et al. (1977) examined the effect of heat stress on the lethal concentration of epichlorohydrin. Groups of 20 male mice (weighing 18-26 g) and 20 male rats weighing (230-270 g) were exposed to epichlorohydrin by inhalation for either 2 hours (mice) or 4 hours (rats). The strains of rodents used were unspecified. Half of the animals in each group were then placed in a heated chamber for 45 minutes at 35°C. The remaining animals were kept at room temperature (18°C). Two similar, nonexposed control groups of mice and rats were either placed in the heated chamber or kept at room temperature. The heated chamber had a relative humidity of 35-50 percent. The authors reported that the rats were more sensitive to heat stress than were the mice. However, care should be used in evaluating this study as the confidence limits of the respective LC_{50} values are large and overlap considerably. The LC_{50} values obtained for epichlorohydrin are shown in Table 8-2. Information on syngergism and antagonism at the physiological level was limited to a few fragmentary animal studies. Epichlorohydrin administered orally in combination with cholesterol affected heart functions and blood lipid levels in rabbits (Lukaneva and Rodinov 1978). Rats that were administered epichlorohydrin by inhalation for 4 hours and subsequently cold-stressed, showed increased bromsulphalein retention (measured 24 hours after treatment) compared with noncold-stressed animals; there were no significant differences between the two groups in body temperature, oxygen demand, kidney weight, urine volume, and total blood protein, urea nitrogen, and serum sulfhydryl levels (Shumskaya et al., 1971). Another study of rats indicated that heat (35°C) enhanced the toxicity of epichlorohydrin (Grigorowa et al. 1977).

TABLE 8-2. THE EFFECT OF HEAT STRESS ON THE LD_{50} OF EPICHLOROHYDRIN IN THE RAT AND MOUSE[a]

Species	Condition	LC_{50} (mg/l)	Confidence Limits
Rat	No heat	2.40	0.87-6.56
	With heat	2.20	0.67-7.18
Mouse	No heat	3.00	1.79-5.02
	With heat	4.00	2.57-6.22

[a] Source: Grigorowa et al. (1977).

9. ECOSYSTEM CONSIDERATIONS

9.1 EFFECTS ON MICROORGANISMS AND PLANTS

9.1.1 Effects on Microorganisms and Lower Plants

There were very few studies available that reported the effects of epichlorohydrin on microorganisms, but all indicate that toxic effects would occur at concentrations in excess of a few milligrams per liter. None of the studies, however, was performed in a closed system with a constant, measured concentration of epichlorohydrin. Moreover, when released into natural habitats, epichlorohydrin would not be expected to persist because of its tendencies to hydrolyze and volatilize.

Toxicity threshold concentrations (i.e., lowest inhibitory concentrations) of epichlorohydrin for *Scenedesmus quadricauda* (green alga), *Microcystis aeruginosa* (blue-green alga), *Entosiphon sulcatum* (flagellated protozoan), and *Pseudomonas putida* (aerobic bacterium) were 5.4, 6.0, 35.0, and 55.0 mg/l, respectively (Bringmann and Kuhn 1976, 1980). All of the species were studied by comparable procedures to determine the minimum toxicant levels that inhibited cell multiplication. Inhibition was measured by turbidimeter for algae and bacteria and by electronic cell counter for protozoa. For each organism, three parallel dilution series were prepared, and the toxicity threshold was estimated graphically by plotting cell numbers (per ml) against log concentration of epichlorohydrin (mg/l). Test durations were 16 hours for bacteria, 72 hours for protozoa, and 168 hours for the two algae. These studies may indicate toxicity thresholds but are of limited usefulness because the epichlorohydrin concentrations were not measured and chemical purity was not specified.

Kolmark and Giles (1955) investigated the mutagenicity of epichlorohydrin to an adenine-requiring strain of the purple fungus, *Neurospora crassa*, and also observed toxic effects. Conidia were treated with 0.15 M epichlorohydrin (13.88 g/l). Survival decreased to 40 percent and 0.7 percent with treatment periods of 45 and 60 minutes, respectively. Chemical purity was not specified, and statistical analysis of the data was not indicated.

Other studies indicate that epichlorohydrin is mutagenic to bacteria and fungi at levels well above a few milligrams per liter (see Section 7.2).

9.1.2 Effects on Higher Plants

The only study available for higher plants reported the effects of treating *Eucalyptus* seeds with epichlorohydrin.

Epichlorohydrin was one of five chemical mutagens used to treat the seeds of three species of *Eucalyptus* (Bandel 1971). Groups of 400 seeds from each species were treated with 0.15 percent (1.5 g/l) and 0.30 percent (3.0 g/l) solutions for 2 or 4 hours each. The treated seeds were then sown in wooden boxes with soil and sterilized manure. Sixty days after sowing, the number of live plants was determined (Table 9-1). Results indicated decreased survival with increasing concentration or exposure period. In addition, *E. citriodora* appeared to be more resistant than the other two species. Chemical purity was not specified, and statistical analyses were not provided.

TABLE 9-1. PERCENT SEEDLING SURVIVAL 60 DAYS AFTER SOWING *EUCALYPTUS* SEEDS TREATED WITH EPICHLOROHYDRIN SOLUTION[a]

Treatment		Percent Survival		
Concentration (%)	Hours	*E. tereticornis*	*E. citriodora*	*E. maculata*
0.00	-	100.00	100.00	100.00
0.15	2	95.07	91.84	98.84
0.15	4	26.91	60.45	41.87
0.30	2	28.08	77.85	33.26
0.30	4	0.00	0.88	0.00

[a] Source: Bandel (1971).

9.2 BIOCONCENTRATION, BIOACCUMULATION, AND BIOMAGNIFICATION

No experimental data were found in the literature on the bioconcentration (direct from the water), bioaccumulation (from food and/or water), or biomagnification (through the food chain) of epichlorohydrin. However, the properties of epichlorohydrin (including its octanol/water partition coefficient (P), susceptibility to aqueous hydrolysis, and volatility) indicate a low likelihood for accumulation in aquatic organisms or food chains.

Several workers have published methods that can be used to estimate bioconcentration. Bioconcentration factors (BCF) can be derived from either water solubility (Chiou et al. 1977, Lu and Metcalf 1975) or log P

(Neely et al. 1974, Veith et al. 1980) (see Appendix D). Log P was estimated to be 0.26 ± 0.04 according to the method of Hansch and Leo (1979) (see Appendix C). The log BCF values for epichlorohydrin estimated by these methods range from -0.032 to 0.968. Log BCF values less than 2 indicate a low bioconcentration potential (Kenaga 1980).

9.3 EFFECTS ON AQUATIC ANIMALS

Limited data were available to indicate the effects on aquatic biota. The only aquatic toxicity studies found in the literature were laboratory tests performed under static conditions, and the epichlorohydrin concentration was measured in only one of them. In assessing the available data, one must consider the compound's environmental fate (see Section 3.5.1). Epichlorodydrin that is released into natural waters is not expected to persist beyond a few days because of its general reactivity and its tendency to hydrolyze and/or volatilize. Additional factors affecting the results of epichlorohydrin toxicity tests relate to experimental conditions (e.g., temperature, pH, water hardness, chemical synergism, dissolved oxygen, and disease) (U.S. EPA 1975). Information on epichlorohydrin, however, is too limited to examine the effects of these parameters.

The acute lethal effects of epichlorohydrin have been reported for four fish and one invertebrate (see Table 9-2). Static median lethal values ranging from 18 to 35 mg/l were reported. In only one of the tests, however, was the actual epichlorohydrin concentration measured. There was no information on subchronic or chronic exposures or flow-through tests found in the literature.

9.3.1 Freshwater Fish

Toxicity information on epichlorohydrin was found for three warm water fish; there was no toxicity information available on cold water fish. In a bluegill study (Dawson et al. 1977), the test fish were obtained from commercial hatcheries and held in 114-liter aquaria for 14 days at 23°C prior to testing. During this period, the fish were fed an unspecified "commercial fish food," treated to prevent disease, and maintained in a minimum water volume of 1 liter/gram of fish. Test fish were selected only from those holding tanks showing less than 5 percent mortality. Aeration was not used during the initial 24-hour test period. Dissolved oxygen was measured daily, and dead fish were counted and removed daily. Toxicant levels were not measured analytically in this static test. The acute toxicity results for bluegill are summarized in Table 9-3. The death rate for the control fish was low at 1.3 percent.

TABLE 9-2. EPICHLOROHYDRIN TOXICITY TO FOUR FISH AND ONE AQUATIC INVERTEBRATE

Species	Temperature (°C)	Test[a]	Toxic Level (mg/l)	No-Effect Level (mg/l)	Comments	Reference
FISH						
FRESHWATER						
Bluegill (Lepomis macrochirus)	23	S,U	96-h LC_{50} = 35	10	Test conducted in well water: pH 7.6-7.9, hardness 55 mg/l as ($CaCO_3$)	Dawson et al. (1977)
Goldfish (Carassius auratus)	20 ± 1	S,M	24-h TL_m = 23[b]	23	Only study with measured epichlorohydrin levels; chemically defined tapwater.	Bridié et al. (1979b)
Ide (Leuciscus idus melanotus)	20 ± 1	S,U	48-h LC_{50} = 24	12	Test conducted in tapwater: pH 7-8 hardness 268 ± 54 mg/l	Juhnke and Lüdemann (1978)
SALTWATER						
Tidewater silverside (Menidia beryllina)	20	S,U	96-h LC_{50} = 18	10	"Instant Ocean" sea salt mix; sp gr = 1.018	Dawson et al. (1977)
INVERTEBRATE						
Waterflea (Daphnia magna)	20	S,U	24-h LC_{50} = 30	20	Test conducted in chlorine-free tapwater at pH 7.6	Bringmann and Kühn (1977)

[a] S = Static; U = Unmeasured Concentrations; M = Measured Concentrations.

[b] Median Tolerance Limit.

Table 9-3. The Acute Toxicity of Epichlorohydrin to Bluegill and Tidewater Silverside Fish[a]

Species	Initial Concentration (mg/l)	Percent Survival After 24 h	48 h	72 h	96 h	Best Fit 96-h LC_{50} (mg/l)
Bluegill (*Lepomis macrochirus*)	56	0	--	--	--	
	42	50	0	--	--	
	37	100	90	80	60	35
	32	100	90	80	70	
	10	100	100	75	75	
Tidewater silverside (*Menidia beryllina*)	32	100	30	0	--	
	18	100	90	70	50	18
	10	90	90	90	90	

[a] Source: Dawson et al. (1977).

The goldfish study (Bridí et al. 1979b) was a static bioassay performed using the methodology published by the American Public Health Association (APHA 1976). The study was done without aeration using tapwater in 25-liter aquaria. The chemical composition of the aged tapwater was determined, and epichlorohydrin concentrations were measured before and after the test.

Juhnke and Ludemann (1978) reported the static acute toxicity of epichlorohydrin to the ide (golden orfe), a species introduced from Europe and established in U.S. waters. The 48-hour LC_{50} value was 24 mg/l; 0 and 100 percent mortality occurred at 12 and 35 mg/l, respectively. The bioassay was conducted according to the method of Mann (1976). Ten fish (0.3 g, 5-7 cm) were exposed for 48 hours to epichlorohydrin (nominal levels) in tapwater (pH 7-8, hardness 268 ± 54 mg/l) at 20 ± 1°C. Although composition of the tapwater was not completely specified, the experimental pH, hardness, and temperature values were within the range of values likely to be found in the natural environment.

9.3.2 Freshwater Invertebrates

In the only available study reporting the effects of epichlorohydrin on an aquatic invertebrate, the 24-hour LC_{50} for *Daphnia magna* was determined to be 30 mg/l (Bringmann and Kuhn 1977). In this static test, no *D. magna* were killed at 20 mg/l, while all were killed at 44 mg/l. The study was conducted in chlorine-free tapwater at 20°C and pH 7.6. Epichlorohydrin levels were not actually measured. Three parallel dilution series were studied using 10 D. magna in each culture vessel. The cessation of swimming was considered to be equivalent to death.

9.3.3 Saltwater Fish

The only study found in the literature on the effects of epichlorohydrin on saltwater biota involved the tidewater silverside, Menidia beryllian, an estuarine fish. Dawson et al. (1977) reported the 96-hour LC_{50} to be 18 mg/l (Table 9-3). Tidewater silversides were obtained from Horsehoe Bay near Sandy Hook, New Jersey. They were acclimated for 14 days in 114-liter aquaria at 20°C and fed minced frozen shrimp. Dilution water was obtained from a well in Passaic, New Jersey, and was the base for a synthetic sea salt medium ("Instant Ocean"). A specific gravity of 1.018 was maintained. Water for testing was prepared 1 day in advance and placed in 19-liter test aquaria. Tidewater silversides (40-100 mm in length) were randomly selected for the assays. Continuous aeration was considered necessary because of the activity and size of the fish. Only nominal toxicant levels were reported for this static test. Mortality counts and LC_{50} values were determined as for the bluegill (Section 9.3.1). The death rate of control fish during the experiment was acceptably low at 3.0 percent.

9.4 SUMMARY

The limited data found on the effects of epichlorohydrin on microorganisms and plants indicate that growth inhibition and toxicity would occur at greater than 5 mg/l. Theoretical estimates of bioconcentration suggest that epichlorohydrin would not accumulate substantially in food chains.

Limited toxicity data for five aquatic animals indicated that exposure to epichlorohydrin concentrations of more than 10 mg/l for 1-4 days would be harmful. In only one of the tests, however, was the actual epichlorohydrin concentration measured. No data were found on the effects of longer exposures.

10. REGULATIONS AND STANDARDS

Epichlorohydrin is regulated under numerous U.S. and foreign statutes. These have been grouped according to the type of activity or medium being controlled.

10.1 OCCUPATIONAL STANDARDS

The current OSHA standard for epichlorohydrin levels in the workplace is 19 mg/m^3 (5 ppm) (29 CFR 1910.1000). This threshold limit value, expressed as an 8-hour time-weighted average (TWA), was based on the known acute health effects to humans from respiratory tract irritation and systemic poisoning. After a comprehensive literature review, NIOSH (1976a) concluded that human exposure risks may include carcinogenesis, mutagenesis, and sterility. NIOSH (1976b) recommended that worker exposure to epichlorohydrin be limited to 0.5 ppm (2 mg/m^3) for a 40-hour workweek, with a ceiling value of 15 ppm (15 minutes). At the time of this report, OSHA had not adopted the lower, NIOSH-recommended TWA. Table 10-1 presents the accepted occupational standards for epichlorohydrin exposure in seven countries.

10.2 FOOD TOLERANCES

FDA permits an epichlorohydrin-derived resin reacted with ammonia to be used as an ion-exchange resin in the treatment of food and potable water (21 CFR 173.25) and use of molecular sieve resins cross-linked with epichlorohydrin for processing foods and production of whey (21 CFR 173.40). Industrial starch (21 CFR 178.3520) and food starch may be cross-linked by treatment with epichlorohydrin (not to exceed 0.3 percent) alone or in conjunction with propylene oxide, acetic anhydride, or succinic anhydride (21 CFR 172.892). Traces of free epichlorohydrin have been found in resins manufactured outside of the United States (NIOSH 1976a).

Various resins of epichlorohydrin can be used in the manufacture of paper and paperboard that will be in contact with dry, aqueous, and fatty foods (21 CFR 175.300, 175.390, and 175.320). In particular, 4,4'-isopropylidene-diphenol-epichlorohydrin resins (minimum molecular weight 10,000) and 4,4'-isopropylidenediphenol-epichlorohydrin thermosetting epoxy resins may be used as articles or components of articles intended for food-related uses (21 CFR 177.1440 and 177.2280). Epichlorohydrin is also regulated by the FDA as a component of adhesives (21 CFR 175.105).

TABLE 10-1. OCCUPATIONAL STANDARDS FOR EPICHLOROHYDRIN

Standard	Country	Level (ppm)
MAC[a]	Netherlands	2.0[b]
MAC	U.S.S.R.	0.26[c]
MAC	Czechoslovakia	0.26[c]
MAC	Federal Republic of Germany	3.6[c]
MAC	German Democratic Republic	1.0[c]
MAC	Rumania	2.6[d]
TWA[e]	U.S.	5.0[f]

[a] Maximum Allowable Concentration

[b] Source: IRPTC (1979).

[c] Sources: Winell (1975), Sram et al. (1980).

[d] Source: Wexler (1971).

[e] Time-weighted average

[f] Source: 29 CFR 1910.1000

10.3 TRANSPORTATION REGULATIONS

Epichlorohydrin transport on both land and water is regulated. The Department of Transportation (DOT) has designated epichlorohydrin as a "hazardous material for the purpose of transportation" (49 CFR 172). This requires container labeling for class 3 poisons as follows:

> EPICHLOROHYDRIN
> POISON! FLAMMABLE!
> SKIN CONTACT CAUSES DELAYED BURNS
>
> Avoid contact with eyes, skin, and clothing.
> Avoid breathing vapor.
> Use only with adequate ventilation.
> Keep away from heat and open flame.
> Keep container closed.
> Do not take internally.
>
> First Aid: In case of skin contact, immediately remove all contaminated clothing, including footwear; wash skin with plenty of water for at least 15 minutes; and call a physician. In case of eye contact, flush eyes with water for 15 minutes and call a physician.

The U.S. Coast Guard under 33 CFR 126, 46 CFR 153, and 46 CFR 151 also has developed safe handling procedures for epichlorohydrin in waterfront areas, in self-propelled vessels, and in unmanned barges. The required warning labels are similar to that developed by DOT (above).

10.4 WATER REGULATIONS

Epichlorohydrin is not regulated under the Safe Drinking Water Act. The Clean Water Act prohibits the discharge of more than 1,000 pounds (454 kg) of epichlorohydrin into navigable waters (40 CFR 116). Discharge at this level may be harmful and must be reported.

10.5 SOLID WASTE REGULATIONS

Under the Resources Conservation Recovery Act, EPA has designated epichlorohydrin as a "hazardous waste" (40 CFR 261). If quantities exceed 100 kg/month, disposal must be in a special landfill. Compliance with the National Pollutant Discharge Elimination System (NPDES) is also required (40 CFR 122).

REFERENCES

Abdel-Sayed, H. W.; Yousef S. H.; Tadros, F. E.; Soliman, N. Z. (1974) The effect of epichlorohydrin to prevent the reaction between DDT lindane oil emulsion and the inside walls of the containers. Agric. Res. Rev. 52:145-161.

Adamek, P.; Peterka, V. (1971) The determination of epichlorohydrin in aqueous solutions in the presence of glycerin, monochlorohydrin and glycidol. Analyst (London) 96:807-809.

Addy, J. K.; Parker, R.E. (1965) The mechanism of epoxide reactions. Part VII. The reactions of 1,2-epoxybutane, 3,4-epoxy-1-butene, 1,2-epoxy-3-chloropropane, and 1,2-epoxy-3-methoxypropane with chloride ion in water under neutral and acidic conditions. J. Chem. Soc. Jan:644-649.

Attman, P. L. (ed); Dittmer, D. S. (ed). (1974) Biology Data Book, v. 3. 2nd ed. Bethesda, MD: Federation of American Societies for Experimental Biology.

American Conference of Governmental Industrial Hygienists. (1979) Documentation of threshold limit values for chemical substances in workroom air. 4th ed. Cincinnati, OH: American Conference of Governmental Industrial Hygienists; pp. 16-17, 34-37.

American Public Health Association. (1976) Standard methods for the examination of water and wastewater. 14th ed. Washington, DC: American Public Health Association; p. 1076.

Ames, B. N.; McCann, J.; Yamasaki, E. (1975) Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity tests. Mutat. Res. 31:347-364.

Andersen, M.; Kiel, P.; Larsen, H.; Maxild, J. (1978) Mutagenic action of aromatic epoxy resins. Nature (London) 276:391-392.

Anderson, G. E.; Liu, C. S.; Holman, H. Y.; Killus, J. P. (1980) Human exposure to atmospheric concentrations of selected chemicals. Appendix A-13: A summary of data on epichlorohydrin. Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Air Quality Planning and Standards.

Andersson, K.; Levin, J. O.; Lindahl, R.; Nilsson, C. A. (1981) Sampling of epichlorohydrin and ethylene chlorohydrin in workroom air using Amberlite XAD-7 resin. Chemosphere 10:143-146.

Anonymous. (1961) Epichlorohydrin (1-chloro-2,3-epoxypropane). Am. Ind. Hyg. Assoc. J. 22:511-513.

Anonymous. (1978) Derailment spills epi. Chem. Week 122:22.

Bandel, G. 1971. Methods for inducing mutations in Eucalyptus. Cienc. Cult. (Sao Paulo) 23:241-246.

Bartsch, H.; Malaveille, C.; Camus, A. M.; Martel-Planche, G.; Brun, G.; Hautefeuille, A.; Sabadie, N.; Barbin, A.; Kuroki, T.; Drevon, C.; Piccoli, C.; Montesano, R.; (1980) Validation and comparative studies on 180 chemicals with *S. typhimurium* strains and V79 Chinese hamster cells in the presence of various metabolizing systems. Mutat. Res. 76:1-50.

Beckwith, A. L. J. (1972) Phosphorous and hypophosphorous acid derived radicals and their reactions - an E.P.R. study. Aust. J. Chem. 25:1887-1905.

Blackford, J. L. (1978) Marketing research report on epichlorohydrin. In: Chemical economics handbook. Menlo Park, CA: SRI International; pp. 642.3021A-642.3022V.

Bridges, B. A. (1978) On the detection of volatile liquid mutagens with bacteria: experiments with dichloros and epichlorohydrin. Mutat. Res. 54:367-371.

Bridié, A. L.; Wolff, C. J. M.; Winter, M. (1979a) BOD and COD of some petrochemicals. Water Res. 13:627-630.

Bridié, A. L.; Wolff, C. J. M.; Winter, M. (1979b) The acute toxicity of some petrochemicals to goldfish. Water Res. 13:623-626.

Briggs, G. G. (1973) A simple relationship between adsorption of organic chemicals and their octanol/water partition coefficients. In: Proceedings of the 7th British insecticide and fungicide conference; pp. 83-85.

Bringmann, G.; Kühn, R. (1976) Comparative results of the harmful effects of water pollutants on bacteria (*Pseudomonas putida*) and blue-green algae (*Microcystis aeruginosa*). GWF Gas Wasserfach: Wasser Abwasser 117:410-413.

Bringmann, G.; Kühn, R. (1980) Comparison of the toxicity thresholds of water pollutants to bacteria, algae, and protozoa in the cell multiplication inhibition test. Water Res. 14:231-241.

Bringmann, G.; Kühn, R. (1977) Findings on the damaging action of water pollutants on *Daphnia magna*. Z. Wasser Abwasser Forsch. 10(5):161-166.

Brönsted, J. N.; Kilpatrick, M.; Kilpatrick, M. (1929) Kinetic studies on ethylene oxides. J. Am. Chem. Soc. 51:428-461.

Bufalini, M. (1971) Oxidation of sulfur dioxide in polluted atmospheres - a review. Environ. Sci. Technol. 5(8):685-700.

Calvert, J. G.; Pitts, J. N., Jr. (1966) Photochemistry. New York, NY: John Wiley & Sons, Inc.; pp. 522-528.

Carbone, P.; Barbata, G.; Margiotta, G.; Tomasino, A.; Granata, G. (1981) Low epichlorohydrin concentrations induce sister chromatid exchanges in human lymphocytes *in vitro*. Caryologia 34:261-266.

Carcinogen Assessment Group, U.S. Environmental Protection Agency. (1980) Preliminary risk assessment of epichlorohydrin. Type I - air program. Prepared for the Office of Air Quality Planning and Standards.

Carpenter, C. P.; Smyth, H. F., Jr.; Pozzani, U. C. (1949) The assay of acute vapor toxicity, and the grading and interpretation of results on 96 chemical compounds. J. Ind. Hyg. Toxicol. 31:343-346.

Castro, C. E.; Bartnicki, E. W. (1968) Biodehalogenation. Epoxidation of halohydrins, epoxide opening, and transhalogenation by a *Flavobacterium* sp. Biochemistry 7:3213-3218.

Chiou, C. T.; Freed, V. H.; Schmedding, D. W.; Kohnert, R. L. (1977) Partition coefficient and bioaccumulation of selected organic chemicals. Environ. Sci. Technol. 11:475-478.

Cooper, E. R.; Jones, A. R.; Jackson, H. (1974) Effects of alpha-chlorohydrin and related compounds on the reproductive organs and fertility of the male rat. J. Reprod. Fertil. 39:379-386.

Cox, C. R. (1972) Regression model and life tables. J. R. Stat. Soc. B 34:187-220.

Crump KS. 1979. Dose-response problems in carcinogenesis. Biometrics 35:157-167.

Crump, K. S.; Watson, W. W. (1979) GLOBAL 79. A Fortran program to extrapolate dichotomous animal carcinogenicity data to low dose. National Institute of Environmental Health Science Contract No. 1-ES-2123.

Crump, K. S.; Howe, R. B. (1983) ADOLL1-83 and ADOLL2-83, FORTRAN programs for implementing a multistage model with a time-dependent dose pattern. Unpublished instructions for use of computer programs.

Crump, K. S.; Howe, R. B. (1984)The multistage model with a time-dependent dose pattern: applications to carcinogenic risk assessment. Risk Analysis 4:163-176.

Crump, K. S.; Guess, H. A.; Deal, L. L. (1977) Confidence intervals and test of hypotheses concerning dose-response relations inferred from animal carcinogenicity data. Biometrics 33:437-451.

Dabney, B. J.; Johnston, R. V.; Quast, J. F.; Park, C. N. (1979) Epichlorohydrin - subchronic studies. III. Cytogenetic evaluation of bone marrow cells from rats exposed by inhalation to epichlorohydrin for four weeks. International Commission for Protection Against Environmental Mutagens and Carcinogens; document no. 128, 15pp.

Daniel, J. W.; Gage, J. C. (1956) The determination of epichlorohydrin in air. Analyst (London) 81:594-598.

Dawson, G. W.; Jennings, A. L.; Drozdowski, D.; Rider, E. (1977) The acute toxicity of 47 industrial chemicals to fresh and saltwater fishes. J. Hazard. Mater. 1:303-318.

Dean, B. J.; Hodson-Walker, G. (1979) An *in vitro* chromosome assay using cultured rat-liver cells. Mutat. Res. 64:329-337.

DePetrocellis, L.; Tortoreto, M.; Paglialunga, S.; Paesani, R.; Airoldi, L.; Ramos Castaneda, E.; Pantarotto, C. (1982) Determination of epichlorodydrin in blood by gas chromatography and selected ion monitoring. J. Chromatogr. 240:218-23.

Dilling, W. L. (1977) Interphase transfer processes. II. Evaporation rates of chloro methanes, ethanes, ethylenes, propanes, and propylene from dilute aqueous solutions. Comparisons with theoretical predictions. Environ. Sci. Technol. 11:405-409.

Dilling, W. L.; Bredeweg, C. J.; Tefertiller, N. B. (1976) Organic photochemistry. Simulated atmospheric photodecomposition rates of methylene chloride, 1,1,1-trichloroethane, trichloroethylene, tetrachloroethylene, and other compounds. Environ. Sci. Technol. 10:351-356.

Dobbs, A. J.; Gilbert, B. C.; Laue, H. A. H.; Norman, R. O. C. (1976) Electron spin resonance studies. Part IL. Radicals derived from oxirans in aqueous solution. J. Chem. Soc. Perkin Trans. 2:1044-1047.

Dobinson, B.; Hofmann, W.; Stark, B. P. (1969) The determination of epoxide groups. Oxford, England: Pergamon Press; pp. 2-61.

Doll, R. (1971) Weibull distribution of cancer. Implications for models of carcinogenesis. J. R. Stat. Soc. A 13:133-166.

Dow Chemical Company. (1980) Epichlorohydrin -- properties, uses, storage, handling. Midland, MI: Dow Chemical Company.

Dripps, R. D.; Eckenhoff, J. E.; Vandam, L. D. (1977) Introduction to anesthesia, the principles of safe practice. 5th ed. Philadelphia, PA: W. B. Saunders Company, pp. 121-123.

Eder, E.; Neudecker, T.; Lutz, D.; Henschler, D. (1980) Mutagenic potential of allyl and allylic compounds: structure-activity relationship as determined by alkylating and direct _in vitro_ mutagenic properties. Biochem. Pharmacol. 29:993-998.

Elmore, J. D.; Wong, J. L.; Laumbach, A. D.; Streips, U. N. (1976) Vinyl chloride mutagenicity via the metabolites chlorooxirane and chloroacetaldehyde monomer hydrate. Biochim. Biophys. Acta 442:405-419.

Enterline, P. E. (1978) Updated mortality in workers exposed to epichlorohydrin. Unpublished reports to Shell Oil Co. (Aug. 31, 1977; July 31, 1978) and letter to R.E. Joyner, Shell Oil Co. (July 31, 1978). Letter from R.E. Joyner, corporate Medical Director, Shell Oil Co., to J.M. Lane, Acting Director, NIOSH (Aug. 7, 1978).

Enterline, P. E. (1980) Cancer in men manufacturing isopropyl alcohol. Report attached to a letter from M.J. Sloan (Nov. 11, 1980), Shell Oil Co., to U.S. Environmental Protection Agency. EPA document control No. OTS-1180-0069.

Enterline, P. E. (1981) Further update of mortality among workers exposed to epichlorohydrin. Report to Shell Oil Co., Jan. 13, 1981. Submitted to U.S. Environmental Protection Agency, Office of Toxic Substances. Docket No. 8EHQ-0878-0230 Supplement.

Enterline, P. E. (1982) Importance of sequential exposure in the production of epichlorohydrin and isopropanol. Ann. NY Acad. Sci. 381:344-349.

Epstein, S. S.; Arnold, E.; Andrea, J.; Bass, W.; Bishop, Y. (1972) Detection of chemical mutagens by the dominant lethal assay in the mouse. Toxicol. Appl. Pharmacol. 23:288-325.

Fakhouri, G.; Jones, A. R. (1979) Epichlorohydrin: metabolism and toxicity in the rat. Aust. J. Pharm. Sci. 8:11-14.

Falco, J. W.; Mulkey, L. A.; Swank, R. R., Jr.; Lipcsei, R. E.; Brown, S. M. (1982) A screening procedure for assessing the transport and degradation of solid waste constitutents in surface and subsurface waters. Environ. Toxicol. Chem. 1:121-134.

Fjellstedt, T. A.; Allen, R. H.; Duncan, B. K.; Jakoby, W. B. (1973) Enzymatic conjugation of epoxides with glutathione. J. Biol. Chem. 248:3702-3707.

Fomin, A. P. (1966) Biological effect of epichlorohydrin and its hygienic significance as an atmospheric pollutant. Gig. Sanit. 31:357-363.

Freuder, E.; Leake, C. D. (1941) The toxicity of epichlorohydrin. Univ. Calif. Berkeley, Publ. Pharmacol. 2:69-78.

Gage, J. C. (1959) The toxicity of epichlorohydrin vapour. Br. J. Ind. Med. 16:11-14.

Gay, B. W., Jr.; Bufalini, J. J. (1971) Nitric acid and the nitrogen balance of irradiated hydrocarbons in the presence of oxides of nitrogen. Environ. Sci. Technol. 5:422-425.

Gillenwater, L. E. (1965) Stream pollution by chemical spills from common carriers. J. Am. Water Works Assoc. 57:201-207.

Gomes, W. R. (1977) Pharmacological agents and male fertility. In: Johnson, A. D.; Gomes, W. R.; Vandemark, N. L., eds. Testis. v. 4. New York, NY: Academic Press; pp. 605-628.

Greve, P. A. (1971) Chemical wastes in the sea: New forms of marine pollution. Science (Washington, DC) 173:1021-1022.

Grigorowa, R.; Gohlke, R.; Rothe, R.; Weigmann, H. J. (1977) MAC value for epichlorohydrin at normal and elevated ambient temperatures. Z. Gesamte Hyg. Ihre Grenzgeb. 23:620-623.

Gusev, M. I.; Minayev, A. A. (1973) Voprosy gigieny atmosfernogo vozdukha i planirovki naselennykh mest [Determination of latent time of reflex reactions of laboratory animals acted upon by toxic agents in atmospheric air.] Uch. Zap. Mosk. Nauchno Issled. Inst. Gig. im F. F. Erismana: 24-26.

Hahn, J. D. (1970) Posttesticular antifertility effects of epichlorohydrin and 2,3-epoxypropanol. Nature (London) 226:87.

Hansch, C.; Leo, A. (1979) Substituent constants for correlation analysis in chemistry and biology. New York, NY: John Wiley & Sons; p. 321.

Hawley, G. G. (ed). (1977) The condensed chemical dictionary. 9th ed. New York, NY: Van Nostrand Reinhold Co.; p. 343.

Hayakawa, T.; Udenfriend, S.; Yagi, H.; Jerina, D. M. (1975) Substrates and inhibitors of hepatic glutathione-s-epoxide transferase. Arch. Biochem. Biophys. 170:438-451.

Heslot, H. (1962) Etude quantitative de reversions biochemiques chez la lerure Schizosaccharomyces pombe par des substances radiomimetiques. Abh. Dtsch. Akad. Wiss. Berlin, K. L. Med. 1:193-228.

Hine, C.; Rowe, V. K.; White, E. R.; Darmer, K. I., Jr.; Youngblood, G. T. (1981) Miscellaneous epoxy compounds. In: Clayton, G. D.; Clayton, F. E., eds. Patty's industrial hygiene and toxicology, 3rd ed., Vol. II-A. New York, NY: John Wiley & Sons; pp. 2148-2154, 2242-2247.

Hushon, J.; Clerman, R.; Small, R.; Sood, S.; Taylor, A.; Thoman, D. (1980) An assessment of potentially carcinogenic, energy-related contaminants in water. McLean, VA: Mitre Corporation; p. 117 MTR-79W171.

International Commission on Radiological Protection (ICRP). (1977) Recommendation of the International Commission on Radiological Protection, adopted Jan. 17, 1977. Oxford, England: Pergamon Press. Publ. no. 26.

Ippen, H.; Mathies, V. (1970) Protracted chemical burns (with special consideration of dermatological damage due to epoxides and propansultone). Berufs Dermatosen 18:144-165.

Jaraczewska, W.; Kaszper, W. (1967) Determination of epichlorohydrin in air. Med. Pr. 18:168-176.

Jirasek, L.; Kalensky J. (1960) Epoxy resins. Prac. Lek. 12:67-74.

John JA; Quast JF; Murray FJ; Henck JW; Calhoun LG; Murray JS; Pilny MK Deacon MS; Crawford AA; and Schwetz BA. 1979. The effects of inhaled epichlorohydrin on the semen of rabbits and on the fertility of male and female rats. Midland, MI: Dow Chemical USA, Toxicology Research Laboratory, Health and Environmental Sciences; unpublished report.

Jones, A. R.; O'Brien, R. W. (1980) Metabolism of three active analogues of the male antifertility agent alpha-chlorohydrin in the rat. Xenobiotica 10:356-370.

Jones, A. R.; Davies, P.; Edwards, K.; Jackson, H. (1969) Antifertility effects and metabolism of alpha- and epichlorohydrins in the rat. Nature (London) 224(5214):83.

Juhnke, I.; Ludemann, D. (1978) Results of the investigation of 200 chemical compounds for acute fish toxicity with the Golden Orfe test. Z. Wasser Abwasser Forsch. 11:161-164.

Kalla, N. R.; Bansal, M. P. (1977) In vivo & in vitro alkylation of testicular cysteine by alpha-chlorohydrin. Ind. J. Exp. Biol. 15:232-233.

Kastenbaum, M. A.; Bowman, K. O. (1970) Tables for determining the statistical significance of mutation frequencies. Mutat. Res. 9:527-549.

Kawabata, A. (1981) Studies on the carcinogenic activity of epichlorohydrin by oral administration in male Wistar rats. Nara Igaku Zasshi 32:270-280.

Kenaga, E. E. (1980) Predicted bioconcentration factors and soil sorption coefficients of pesticides and other chemicals. Ecotoxicol. Environ. Saf. 4:26-38.

Kenaga, E. E.; Goring, C. A. I. (1980) Relationship between water solubility, soil sorption, octanol-water partitioning, and concentration of chemicals in biota. In: Eaton, J. G.; Parrish, P. R.; Hendricks, A. C., eds. Aquatic toxicology. Philadelphia, PA: American Society for Testing and Materials; pp. 78-115; ASTM STP 707.

Kilian, D. J.; Pullin, T. G.; Conner, T. H.; Legator, M. S.; Edwards, H. N. (1978) Mutagenicity of epichlorohydrin in the bacterial assay system: Evaluation by direct in vitro activity and in vivo activity of urine from exposed humans and mice. Mutat. Res. 53:72.

Kirkhart, B. (1981) Micronucleus test on 21 compounds. In: deSerres FJ, Ashby J, eds. Evaluation of short-term tests for carcinogens. Amsterdam, The Netherlands: Elsevier/North Holland; pp. 698-704.

Knaap, A. G. A. C; Voogd, C. E.; Kramers, P. G. N. (1982) Comparison of the mutagenic potency of 2-chloroethanol, 2-bromoethanol, 1,2-epoxybutane epichlorohydrin and glycidaldehyde in Klebsiella pneumoniae, Drosophila melanogaster and L5178Y mouse lymphoma cells. Mutat. Res. 101:199-208.

Kolmark, G.; Giles, N. H. (1955) Comparative studies of monoepoxides as inducers of reverse mutations in Neurospora. Genetics 40:890-902.

Konishi, T.; Kawabata, A.; Denda, A.; Ikeda, T.; Katada, H.; Maruyama, H.; Higashiguchi, R. (1980) Forestomach tumors induced by orally administered epichlorohydrin in male Wistar rats. Gann 71:922-923.

Kotin, P.; Falk, H. L. (1963) Organic peroxides, hydrogen peroxide, epoxides, and neoplasia. Radiat. Res. Supp. 3:193-211.

Kremneva, S. N. (1960) Investigation of toxicity of epichlorohydrin and dichlorohydrin. Pr. Toksikol. Moscow Sb. 123-131.

Kremneva, S. N.; Tolgskaya, M. S. (1961) Toxicology of epichlorohydrin. Toksikol. Nov. Prom. Khim. Veshchestv. 2:28-41.

Kucerova, M.; Polivkova, Z. (1976) Banding technique used for the detection of chromosomal aberrations induced by radiation and alkylating agents TEPA and epichlorohydrin. Mutat. Res. 34:279-290.

Kucerova, M.; Polivkova, Z.; Sram, R.; Matousek, V. (1976) Mutagenic effect of epichlorohydrin. I. Testing of human lymphocytes in vitro in comparison with TEPA. Mutat. Res. 34:271-278.

Kucerova, M.; Zhurkov, V. S.; Polivkova, Z.; Ivanova, J. E. (1977) Mutagenic effect of epichlorohydrin. II. Analysis of chromosomal aberrations in lymphocytes of persons occupationally exposed to epichlorohydrin. Mutat. Res. 48:355-360.

Kwart, H.; Goodman, A. L. (1960) The acid-catalyzed ring opening of epichlorohydrin. J. Am. Chem. Soc. 82:1947-1949.

Lambert, D.; Lacroix, M.; Ducombs, G.; Journet, F.; Chapuis, J. L. (1978) Cutaneous allergy to epichlorohydrin. Ann. Dermatol. Venereol. 105:521-525.

Laskin, S.; Sellakumar, A. R.; Kuschner, M.; Nelson, N.; La Mendola, S.; Rusch, G. M.; Katz, G. V.; Dulak, N. C.; Albert, R. E. (1980) Inhalation carcinogenicity of epichlorohydrin in noninbred Sprague-Dawley rats. JNCI J. Natl. Cancer Inst. 65:751-755.

Laumbach, A. D.; Lee, S.; Wong, J.; Streips, U. N. (1977) Studies on the mutagenicity of vinyl chloride metabolites and related chemicals. In: Proceedings of the 3rd international symposium on the prevention and detection of cancer 1:155-170.

Lawrence, W. H.; Autian, J. (1972) Possible toxic effects from inhalation of dental ingredients by alteration of drug biologic half-life. J. Dent. Res. 51:878.

Lawrence WH; Malik M; Turner JE; Autian J. (1972) Toxicity profile of epichlorohydrin. J. Pharm. Sci. 61:1712-1717.

Lawrence, W. H.; Malik, M.; Autian, J. (1974) Development of a toxicity evaluation program for dental materials and products. II. Screening for systemic toxicity. J. Biomed. Mater. Res. 8:11-34.

le Noble, W. J.; Duffy, M. (1964) The effect of pressure on the rates of acid-catalyzed hydrolysis of trimethylene oxide and of epichlorohydrin. J. Phys. Chem. 68:619-622.

Long, F. A.; Pritchard, J. G. (1956) Hydrolysis of substituted ethylene oxides in H_2O solutions. J. Am. Chem. Soc. 78:2663-2667.

Lowenheim, F. A.; Moran, M. K. (1975) Faith, Keyes, and Clark's industrial chemicals, 4th ed. New York, NY: John Wiley & Sons; pp. 335-338.

Lu, P-Y.; Metcalf, R. L. (1975) Environmental fate and biodegradability of benzene derivatives as studied in a model aquatic ecosystem. EHP Environ. Health Perspect. 10:269-284.

Lukaneva, A.M.; Rodionov, G. A. (1978) Study of the influence of polychlorocamphene and epichlorohydrin on the development of experimental atherosclerosis. Fiziol. (Kiev) 24:371-377.

Mackey, B. E.; MacGregor, J. T. (1979) The micronucleus test: statistical design and analysis. Mutat. Res. 64:195-204.

Mann, H. (1976) Fischtest mit Goldorfen zur vergleichenden Prüfung der akuten Toxizität von Wasserinhaltsstöffen und Abwassern-Praktische Erfahrungen aus drei Ringtesten. Z. Wasser Abwasser Forsch. 9:103-109.

Mantel, N.; Schneiderman, M. A. (1975) Estimating "safe" levels, a hazardous undertaking. Cancer Res. 35:1379-1386.

Marks, T. A.; Gerling, F. S.; Staples, R. E. (1982) Teratogenic evaluation of epichlorohydrin in the mouse and rat and glycidol in the mouse. J. Toxicol. Environ. Health 9:87-96.

Mashford, M.; Jones, A. R. (1978) The antifertility action of alpha-chlorohydrin: Enzyme inhibition by alpha-chlorohydrin phosphate. Experientia 34:1267-1268.

Massiot, G.; Levy, J. (1981) Une reaction in attendue de l'epichlorohydrine avec les amines. Bull. Soc. Chim. France (11-12):II-463 - II-467.

Milby, T. H.; Whorton, D. (1980) Epidemiological assessment of occupationally related chemically induced sperm count suppression. JOM J. Occup. Med. 22:77-82.

Milby, T. H.; Whorton, M. D.; Stubbs, H. A.; Ross, C. E.; Joyner, R. E.; Lipshultz, L. I. 1981. Testicular function among epichlorohydrin workers. Br. J. Ind. Med. 38:372-377.

Moore-Brown, M. M.; Clive, D. (1979) The L5178Y/TK$^{+/-}$ mutagen assay system: In situ results. Banbury Rep. 2:71-88.

National Institute for Occupational Safety and Health. (1976a) Criteria for a recommended standard....Occupational exposure to epichlorohydrin. Washington, DC: Department of Health, Education, and Welfare. DHEW Publ. NIOSH 76-206.

National Institute for Occupational Safety and Health. (1976b) A recommended standard for occupational exposure to epichlorohydrin. Washington, DC: Department of Health, Education, and Welfare.

National Institute for Occupational Safety and Health. (1978) Current Intelligence Bulletin 30: Epichlorohydrin. Washington, DC: Department of Health, Education and Welfare DHEW Publication No. 79-105.

Neeley, W. B.; Branson, D. R.; Blau, G. E. (1974) Partition coefficient to measure bioconcentration potential of organic chemicals in fish. Environ. Sci. Technol. 8:1113-1115.

Norppa, H.; Hemminki, K.; Sorsa, M.; Vainio, H. (1981) Effect of monosubstituted epoxides on chromosome aberrations and SCE in cultured human lymphocytes. Mutat. Res. 91:243-250.

Pallade, S.; Dorobantu, M.; Rotaru, G.; Gabrielescu, E. (1967) Experimental studies of intoxication by epichlorohydrin. Arch. Mal. Prof. Med. Trav. Secur. Soc. 28:505-516.

Perry, P.; Wolff, S. (1974) New Giemsa method for the differential staining of sister chromatids. Nature (London) 251:156-158.

Petty, W. L.; Nichols, P. L., Jr. (1954) Rate of addition of the nitrate ion to the ethylene oxide ring. J. Am. Chem. Soc. 76:4385-4389.

Picciano, D. (1979) Cytogenetic investigation of occupational exposure to epichlorohydrin. Mutat. Res. 66:169-173.

Pilny, M. K.; Lederer, T. S.; Murray, J. S.; Deacon, M. S.; Hanley, T. R.; Quast, J. F.; and John, J. A. (1979) Epichlorohydrin--subchronic studies. IV. The effects of maternally inhaled epichlorohydrin on rat and rabbit embryonal and fetal development. Midland, MI: Dow Chemical USA, Toxicology Research Laboratory, Health and Environmental Sciencies; unpunlished report.

Planche, G.; Croisy, A.; Malaveille, C.; Tomatis, L.; Bartsch, H. (1979) Metabolic and mutagenicity studies on DDT and 15 derivatives. Detection of 1,1-bis(p-chlorophenyl)-2,2-dichloroethane and 1,1-bis(p-chlorophenyl)-2,2,2-trichloroethyl acetate (kelthane acetate) as mutagens in Salmonella typhimurium and of 1,1-bis(p-chlorophenyl) ethylene oxide, a likely metabolite, as an alkylating agent. Chem. Biol. Interact. 25:157-175.

Pritchard, J. G.; Long, F. A. (1956) Kinetics and mechanisms of the acid-catalyzed hydrolysis of substituted ethylene oxides. J. Am. Chem. Soc. 78:2667-2670.

Pritchard, J. G.; Siddiqui, I. A. (1973) Activation parameters and mechanism of the acid-catalyzed hydrolysis of epoxides. J. Chem. Soc. Perkin Trans. 2 4:452-457.

Quast, J. F.; Henck, J. W.; Postma, B. J.; Schuetz, D. J.; McKenna, M. J. (1979a) Epichlorohydrin-- Subchronic studies. I. A 90-day inhalation study in laboratory rodents (Fischer 344 rats, Sprague-Dawley rats and B6C3F1 mice). Midland, MI: Dow Chemical U.S.A.

Quast, J. F; Lederer, T. S.; Postma, B. J.; Scheutz, D. J.; John, J. A.; McKenna, M. J. (1979b) Epichlorohydrin--Subchronic studies. II. A 12-day inhalation study in laboratory rodents (Fischer 344 rats, Sprague-Dawley rats and B6C3F1 mice). Midland, MI: Dow Chemical U.S.A.

Rapoport, I. A. (1948) The effect of ethylene oxide, glycide and glycol on genetic mutations. Dokl. Akad. Nauk SSR 60:469-472.

Riesser, G. H. (1978) Epichlorohydrin. In: Kirk-Othmer encyclopedia of chemical technology. V. 5. New York, NY: John Wiley & Sons; pp. 858-864.

Ross, W. C. J. (1950) The reactions of certain epoxides in aqueous solutions. J. Chem. Soc. 3:2257-2272.

Rotaru, G.; Pallade, S. (1966) Experimental studies of histopathological features in acute epichlorohydrin toxicity. Morfol. Norm. Patol. 11:155-164.

Rutledge, J. E.; Islam, M. N. (1973) Canning and pH stability of epichlorohydrin- treated parboiled rice. J. Agric. Food Chem. 21:458-460.

Sax, N. I., ed. (1975) Dangerous properties of industrial materials. 4th ed. New York, NY: Van Nostrand Reinhold Co.; p. 717.

Schultz, V. C. (1964) Fettleber und chronisch-asthmoide Bronchitis nach Inhalation eines Farbenlosungsmittels (Epichlorohydrin). Dtsch. Med. Wochenschr. 89:1342-1344.

Shell Chemical Company. (1969) Epichlorohydrin. Shell Oil Co., Industrial Chemicals Division; technical bulletin no. IC-69-16.

Shellenberger, R. J.; McClimans, C. D.; Ott, M. G.; Flake, R. E.; Daniel, R. L. (1979) An evaluation of the mortality experience of employees with potential for exposure to epichlorohydrin. Submitted to U.S. Department of Labor, OSHA Docket No. H-100. Unpublished.

Shell Oil Company. (1978) Testicular function among ECH workers at Shell Deer Park and Norco facilities. Shell Oil Co. 22 pp.

Sherman, S.; Gannon, J.; Buchi, G.; Howell, W. R. (1980) Epoxy resins. In: Kirk-Othmer encyclopedia of chemical technology. 3rd ed. V. 9 New York, NY: John Wiley & Sons; pp. 267-290.

Shumskaya, N. I.; Karamzina, N. M. (1966) An evaluation of the functional state of rat kidneys during poisoning by industrial substances. Toksikol. Nov. Prom. Khim. Veshchestv. 8:14-27.

Shumskaya, N. I.; Karamzina, N. M.; Savina, M. Y. (1971) Evaluating the sensitivity of integral and specific parameters in acute epichlorohydrin intoxication. Toksikol. Nov. Prom. Khim. Veshchestv. 12:33-44.

Shvets, V. F.; Aleksanyan, L. V. (1973) Kinetics of the hydrolysis of 1-chloro-2,3-epoxy propane in aqueous carbon dioxide salt solutions. Zh. Org. Khim. 10:2027-2030.

Smith, F. A.; Langvardt, P. W.; Young, J. D. (1979) Pharmacokinetics of epichlorohydrin (EPI) administered to rats by gavage or inhalation. Midland, MI: Dow Chemical USA, Toxicology Research Laboratory.

Smyth, H. F., Jr.; Carpenter, C. P. (1948) Further experience with the range finding test in the industrial toxicology laboratory. J. Ind. Hyg. Toxicol. 30:63-68.

Smyth, H. F., Jr.; Carpenter, C. P.; Weil, C. S.; Pozzani, U. C.; Streigel, J. A. (1962) Range-finding toxicity data, List VI. Ind. Hyg. J. 23:95-96.

Sora, S.; Zelaschi, D.; Ronzio, E. (1979) Activity of epichlorohydrin on different genetic systems of Saccharomyces cerevisiae. Atti Assoc. Genet. Ital. 24:274-276.

Sram, R. J.; Cerna, M.; Kucerova, M. (1976) The genetic risk of epichlorohydrin as related to the occupational exposure. Biol. Zentralbl. 95:451-462.

Sram, R. J.; Tomatis, L.; Clemmesen, J.; Bridges, B. A. (1981) An evaluation of the genetic toxicity of epichlorohydrin. Mutat. Res. 87:299-319.

Sram, R. J.; Zudova, Z.; Kuleshov, N. P. (1980) Cytogenetic analysis of peripheral lymphocytes in workers occupationally exposed to epichlorohydrin. Mutat. Res. 70:115-120.

SRI International. (1981) Directory of Chemical Producers. Menlo Park, CA: SRI International.

Stolzenberg, S. J.; Hine, C. H. (1979) Mutagenicity of halogenated and oxygenated three-carbon compounds. J. Toxicol. Environ. Health 5:1149-1158.

Suskov, I. I.; Sazonova, L. A. (1982) Cytogenetic effects of epoxy, phenol-formaldehyde and polyvinylchloride resins in man. Mutat. Res. 104:137-140.

Swan, J. D. (1954) Determination of epoxides with sodium sulfite. Anal. Chem. 26:878-880.

Tassignon, J. P.; Bos, G. D.; Craigen, A. A.; Jacquet, B.; Kueng, H. L.; Lanouziere-Simon, C.; Pierre, C. (1983) Mortality in a European cohort occupationally exposed to epichlorohydrin. Unpublished.

Thorgeirsson, A.; Fregert, S. (1977) Allergenicity of epoxy resins in the guinea pig. Acta Derm. Venereol. 57:253-256.

Thurman, G. B.; Simms, B. G.; Goldstein, A. L.; Kilian, D. J. (1978) The effects of organic compounds used in the manufacture of plastics on the responsivity of murine and human lymphocytes. Toxicol. Appl. Pharmacol. 44:617-641.

Tsuchimoto, T.; Matter, B. E. (1981) Activity of coded compounds in the micronucleus test. In: de Serres, F. J.; Ashby, J. eds. Evaluation of short-term tests for carcinogens. Amsterdam, The Netherlands: Elsevier/ North Holland, pp. 705-711.

U.S. Environmental Protection Agency. (1974) Addendum to development document for proposed effluent limitations guidelines and new source performance standards for the synthetic resins. Washington, DC: U.S. Environmental Protection Agency, Office of Water and Hazardous Materials; pp. 17-24. EPA report no. EPA-440/1-74/036-a.

U.S. Environmental Protection Agency. (1975) Methods for acute toxicity tests with fish, macro-invertebrates, and amphibians. Corvallis, OR: U.S. Environmental Protection Agency, Office of Research and Development; EPA report no. EPA-660/3-75-009.

U.S. Environmental Protection Agency. (1978) Region III. Field investigation, emergency response action, and hydrogeological assistance at Chessie system derailment and spill of epichlorohydrin, Point Pleasant, WV, January 23-39, 1978. Wheeling Field Office.

U.S. Environmental Protection Agency. (1980) Fate of toxic and hazardous materials in the air environment. Research Triangle Park: U.S. Environmental Protection Agency, Office of Research and Development; EPA report no. EPA-600/53-80-084.

U.S. Environmental Protection Agency. (1983) TSCA--plant and production (TSCAPP). CICIS - Chemical Information Tape. EPA/DF-80/005.

Van Duuren, B. L.; Katz, C.; Goldschmidt, B. M. (1972a) Direct-acting alkylating carcinogens--chloro ethers and related compounds. Toxicol. Appl. Pharmacol. 22:279-280.

Van Duuren, B. L.; Katz, C.; Goldschmidt, B. M.; Frenkel, K.; Livak, A. (1972b) Carcinogenicity of halo-ethers. II. Structure-activity relationships of analogs of bis(chloromethyl)ether. JNCI J. Natl. Cancer Inst. 48:1431-1439.

Van Duuren, B. L.; Goldschmidt, B. M.; Katz, C.; Seidman, I.; Paul, J. S. (1974) Carcinogenic activity of alkylating agents. JNCI J. Natl. Cancer Inst. 53:695-700.

van Esch, G. J. (1982) Induction of preneoplastic lesions in the forestomach of rats after oral administration of 1-chloro-2,3-epoxpropane. Preliminary report no. 627805-005.

van Lierop, J. B. H. (1978) Simple and rapid determination of epichlorohydrin at the lower parts per billion level by gas chromatography-mass fragmentography. J. Chromatogr. 166:609-610.

Vashishat, R. K.; Vasudeva, M.; Kakar, S. N. (1980) Induction of mitotic crossing over, mitotic gene conversion and reverse mutation by epichlorohydrin in Saccharomyces cerevisiae. Indian J. Exp. Biol. 18:1337-1338.

Veith, G. D.; Macek, K. J.; Petrocelli, S. R.; Carroll, J. (1980) An evaluation of using partition coefficients and water solubility to estimate bioconcentration factors for organic chemicals in fish. In: Eaton, J. G.; Parrish, P. R.; Hendricks, A. C., eds. Aquatic toxicology. Philadelphia, PA: American Society for Testing and Materials; pp. 116-129 ASTM STP 707.

Venable, J. R.; McClimans, C. D.; Flake, R. E.; Dimick, D. B. (1980) A fertility study of male employees engaged in the manufacture of glycerine. JOM J. Occup. Med. 22:87-91.

Verschueren, K. (1977) Handbook of environmental data on organic chemicals. New York, NY: Van Nostrand Reinhold Co.; pp. 293-294.

Vogel, E.; Schalet, A.; Lee, W. R.; Wurgler, F. (1981) Mutagenicity of selected chemicals in Drosophila. In: de Serres, F. J., ed. Comparative Mutagenesis.

Voogd, C. E. (1973) Mutagenic action of epoxy compounds and several alcohols. Mutat. Res. 21:52-53.

Voogd, C. E.; van der Stet, J. J.; Jacobs, J. J. J. A. A. (1981) The mutagenic action of aliphatic epoxides. Mutat. Res. 89:269-282.

Weast, R. C., ed. (1978) CRC handbook of chemistry and physics, 59th ed. West Palm Beach, FL: CRC Press Inc.; p. C-447.

Weigle, W. W.; Plotnick, H. B.; Conner, W. L. (1978) Tissue distribution and excretion of ^{14}C-epichlorohydrin in male and female rats. Res. Commun. Chem. Pathol. Pharmacol. 20:275-288.

Weil, C. S.; Condra, N.; Hann, C.; Streigel, J. A. (1963) Experimental carcinogenicity and acute toxicity of respresentative epoxides. Am. Ind. Hyg. Assoc. J. 24:305-325.

Westergaard, M. (1957) Chemical mutagenesis in relation to the concept of the gene. Experientia 13:224-234.

Wexler, B. (1971) Determination of epichlorohydrin contamination in an industrial facility for the manufacturing of epoxy resins. Mater. Plast. (Bucharest) 8:322-323.

White, A. D. (1980) In vitro induction of sister chromatid exchange in human lymphocytes by epichlorohydrin with and without metabolic activation. Mutat. Res. 78:171-176.

Winell, M. (1975) An international comparison of hygienic standards for chemicals in the work environment. Ambio 4:34-36.

Wurgler, F. E.; Graf, U. (1981) Mutagenic activity of ten coded compounds in the Drosophila sex-linked recessive lethal assay. In: de Serres, F. J., Ashby, J., eds. Evaluation of short-term tests for carcinogens. Amsterdam, The Netherlands Elsevier/ North Holland; pp. 666-672.

Appendix A. Evaporation Rate of Epichlorohydrin Calculated According to the Method of Dilling (1977)

Equations:

1. $H = (16.04)(P)(M)/(T)(S)$

2. $K_1 = 221.1/[(1.042/H] + 100](M)^{0.5}$

3. $\text{Half-life (days)} = (0.6931/K_1)(d/1{,}440)$

where:

H = Henry's law constant
P = vapor pressure in mmHg at 20° C
M = gram molecular weight of the solute
T = temperature in °K (20° C = 293° K)
S = solubility of the solute in water in mg/l (ppm) at 20° C
K_1 = overall liquid exchange constant in cm/min
d = solution depth in cm

Calculations:

$H = (16.04)(12)(92.53)/(293)(60{,}000) = 1.01 \times 10^{-3}$

$K_1 = 221.1/[(1.042/0.00101) + 100](92.53)^{0.5} = 2.03 \times 10^{-2}$

Half-life (solution depth of 6.5 cm) = (0.6931/0.0203)(6.5/1,440) = 0.15 days
Half-life (solution depth of 100 cm) = (0.6931/0.0203)(100/1,440) = 2.37 days

Appendix B. Soil Adsorption Coefficient (K_{oc}) and Soil Organic Matter/Water Partition Coefficient (Q)

Equations

1. $\log K_{oc} = 3.64 - 0.55 (\log WS) \pm 1.23$ orders of magnitude (Kenaga and Goring 1980)

2. $\log Q = 0.618 - 0.524 (\log P)$ (Briggs 1973)

where:

WS = water solubility

P = octanol/water partition coefficient

log WS	log P	K_{oc}	Q
4.78	0.26	$10.28 \times 10^{\pm 1.23}$	5.68
4.82	0.26	$9.76 \times 10^{\pm 1.23}$	5.68

Appendix C. Calculation of the Log Octanol/Water Partition Coefficient (log P) by the Method of Hansch and Leo (1979)

The π-constant system was used for calculating log octanol/water partition coefficients of epichlorohydrin. Propylene oxide was used as a parent molecule and properly restructured. The appropriate π-constant value was used together with its "uncertainty units." The π-constant is an indication of hydrophobicity and is additive. Relative to hydrogen, a positive value indicates that the substituent favors the octanol phase, whereas a negative value indicates the water phase is favored.

$$\log P = \log P\ CH_3\text{-}CH\text{-}CH_2 + Cl$$

$$= -0.13 + 0.39\ [\pm 0.04]^*$$

$$= 0.26\ [\pm 0.04]^*$$

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[*] Uncertainty units.

Appendix D. Bioconcentration Factors Calculated for Epichlorohydrin by Four Methods

1. log BCF = 0.124 + 0.542 (log P) (Neely et al. 1974) = 0.265

2. log BCF = 0.23 + 0.76 (log) (Veith et al. 1980) = -0.032

3. log BCF = 3.995 - 0.3891 (log WS) (Lu and Metcalf 1975) = 0.968
   (WS = 6.0×10^7 ppb)

4. log BCF = 3.41 - 0.508 (log WS) (Chiou et al. 1977) = 0.458
   (WS = 6.48×10^5 μmole/1)

where:

BCF = bioconcentration factor

P = octanol/water partition coefficient

WS = water solubility

APPENDIX E. COMPARISON OF RESULTS BY VARIOUS EXTRAPOLATION MODELS

The estimates of unit risk from animals presented in the body of this document are all calculated by the use of the linearized multistage model. The reasons for its use have been detailed therein. Essentially, it is part of a methodology that estimates a conservative linear slope at low extrapolations doses and is consistent with the data at all dose levels of the experiment. It is a nonthreshold model holding that the upper limit of risk predicted by a linear extrapolation to low levels of the dose-response relationshop is the most plausible upper limit for the risk.

Other models have also been used for risk extrapolation. Three nonthreshold models are presented here: the one-hit, the log-probit, and the Weibull. The one-hit model is characterized by a continuous downward curvature but is linear at low doses. It can be considered the linear form or first stage of the multistage model because of its functional form. Because of this and its downward curvature, it will always yield estimates of low-level risk that are at least as large as those of the multistage model. Further, whenever the data can be fitted adequately by the one-hit model, estimates from the two procedures will be comparable.

The other two models, the log-probit and the Weibull, are often used to fit toxicological data in the observable range, because of their general "S" curvature. The low-dose upward curvatures of these two models usually yield lower low-dose risk estimates than those of the one-hit or multistage models.

The log-probit model was originally proposed for use in problems of biological assay, such as the assessment of potency of toxicants and drugs, and is generally used to estimate such values as percentile lethal dose or percentile effective dose. Its development was strictly empirical in that it was observed that several log dose-response relationships followed the cumulative normal probability distribution function, Φ. In fitting the cancer bioassay data, assuming an independent background, this becomes:

$$P(D;a,b,xc) = c + (1-c)\Phi\ (a+b\log_{10} D) \quad a,b > 0 \leq c < 1$$

where P is the proportion responding at dose D, c is an estimate of the background rate, a is an estimate of the standardized mean of individuals tolerances, and b is an estimate of the log dose-probit response slope.

The one-hit model arises from the theory that a single molecule of a carcinogen has a probability of transforming a single noncarcinogenic cell into a carcinogenic one. It has the probability distribution function:

$$P(D;a,b) = 1-\exp-(a+bd) \quad a,b > 0$$

where a and b are the parameter estimates. The estimate a represents the background or zero dose rate, and the parameter estimated by b represents the linear component or slope of the dose-response model. In discussing the added risk over background, incorporation of Abbott's correction leads to

$$P(D;b) = 1-\exp-(bd) \quad b > 0$$

Finally, a model from the theory of carcinogenesis arises from the multihit model applied to multiple target cells. This model has been termed here the Weibull model. It is of the form

$$P(D;b,k) = 1-\exp-(bd^k) \quad b,k > 0$$

For the power of dose only, the restriction k > 0 has been placed on this model. When k > 1, this model yields low-dose estimates of risks usually significantly lower than either the linear multistage or one-hit models, which are linear at low doses. All three of these models usually project risk estimates significantly higher at the low exposure levels than those obtained with the log-probit model.

The estimates of added risk for low doses for the above models are given in Table E-1 for the epichlorohydrin drinking water study (Konishi et al., 1980). Both maximum likelihood estimates and 95 percent upper confidence limits are presented. Since all models estimate the background rate as 0, there is no need to incorporate Abbott's correction for independent background rate.

The results (Table E-1) show, in order of descending risk, the one-hit, multistage, Weibull, and log-probit models. The best fit of the data with the multistage model is a cubic with zero linear component, which accounts for its nonlinear behavior at low doses.

TABLE E-1. ESTIMATES OF EPICHLOROHYDRIN LOW-DOSE RISK IN MALE WISTAR RATS DERIVED FROM FOUR DIFFERENT MODELS

Dose	Maximum likelihood estimates of additional risks				95 percent upper confidence limit of additional risks			
	Multistage model	One-hit model	Weibull model	Log-probit model	Multistage model	One-hit model	Weibull model	Log-probit model
0.1 ug/L	0	2.1×10^{-8}	0	0	2.8×10^{-8}	3.4×10^{-8}	0	0
1 ug/L	0	2.1×10^{-7}	0	0	2.8×10^{-7}	3.4×10^{-7}	0	0
10 ug/L	1.4×10^{-17}	2.1×10^{-6}	0	0	2.8×10^{-6}	3.4×10^{-6}	0	0
100 ug/L	2.6×10^{-14}	2.1×10^{-5}	1.1×10^{-14}	0	2.8×10^{-5}	3.4×10^{-5}	2.1×10^{-13}	0
1 mg/L	2.6×10^{-11}	2.1×10^{-4}	1.3×10^{-11}	0	2.8×10^{-4}	3.4×10^{-4}	2.0×10^{-10}	0

SOURCE: Konishi et al., 1980.

APPENDIX F

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER CLASSIFICATION SYSTEM FOR THE EVALUATION OF THE CARCINOGENIC RISK OF CHEMICALS TO HUMANS*

ASSESSMENT OF EVIDENCE FOR CARCINOGENICITY FROM STUDIES IN HUMANS

Evidence of carcinogenicity from human studies comes from three main sources:

1. Case reports of individual cancer patients who were exposed to the chemical or process.
2. Descriptive epidemiological studies in which the incidence of cancer in human populations was found to vary in space or time with exposure to the agents.
3. Analytical epidemiological (case-control and cohort) studies in which individual exposure to the chemical or group of chemicals was found to be associated with an increased risk of cancer.

Three criteria must be met before a causal association can be inferred between exposure and cancer in humans:

1. There is no identified bias which could explain the association.
2. The possibility of confounding has been considered and ruled out as explaining the association.
3. The association is unlikely to be due to chance.

In general, although a single study may be indicative of a cause-effect relationship, confidence in inferring a causal association is increased when several independent studies are concordant in showing the association, when the association is strong, when there is a dose-response relationship, or when a reduction in exposure is followed by a reduction in the incidence of cancer.

The degrees of evidence for carcinogenicity from studies in humans are categorized as:

1. Sufficient evidence of carcinogenicity, which indicates that these is a causal relationship between the agent and human cancer.

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*Adapted from International Agency for Research on Cancer Monographs Supplement 4, Evaluation of the Carcinogenic Risk of Chemicals to Humans, 1982, pp. 11-14.

2. Limited evidence of carcinogenicity, which indicates that a causal interpretation is credible, but that alternative explanations, such as chance, bias, or confounding, could not adequately be excluded.

3. Inadequate evidence, which indicates that one of three conditions prevailed: (a) there were few pertinent data; (b) the available studies, while showing evidence of association, did not exclude chance, bias, or confounding; (c) studies were available which do not show evidence of carcinogenicity.

ASSESSMENT OF EVIDENCE FOR CARCINOGENICITY FROM STUDIES IN EXPERIMENTAL ANIMALS

These assessments are classified into four groups:

1. Sufficient evidence of carcinogenicity, which indicates that there is an increased incidence of malignant tumors: (a) in multiple species or strains; or (b) in multiple experiments (preferably with different routes of administration or using different dose levels); or (c) to an unusual degree with regard to incidence, site or type of tumor, or age at onset. Additional evidence may be provided by data on dose-response effects, as well as information from short-term tests or on chemical structure.

2. Limited evidence of carcinogenicity, which means that the data suggest a carcinogenic effect but are limited because: (a) the studies involve a single species, strain, or experiment; or (b) the experiments are restricted by inadequate dosage levels, inadequate duration of exposure to the agent, inadequate period of follow-up, poor survival, too few animals, or inadequate reporting; or (c) the neoplasms produced often occur spontaneously and, in the past, have been difficult to classify as malignant by histological criteria alone (e.g., lung and liver tumors in mice).

3. Inadequate evidence, which indicates that because of major qualitative or quantitative limitations, the studies cannot be interpreted as showing either the presence or absence of a carcinogenic effect; or that within the limits of these tests used, the chemical is not carcinogenic. The number of negative studies is small, since, in general, studies that show no effect are less likely to be published than those suggesting carcinogenicity.

4. No data indicate that data were not available to the Working Group.

The categories, sufficient evidence and limited evidence, refer only to the strength of the experimental evidence that these chemicals are carcinogenic and not to the extent of their carcinogenic activity nor to the mechanism involved. The classification of any chemical may change as new information becomes available.

EVALUATION OF CARCINOGENIC RISK TO HUMANS

At present, no objective criteria exist to interpret data from studies in experimental animals or from short-term tests directly in terms of human risk. Thus, in the absence of sufficient evidence from human studies, evaluation of the carcinogenic risk to humans was based on consideration of both the epidemiological and experimental evidence. The breadth of the categories of evidence defined above allows substantial variation within each. The decisions reached by the Working Group regarding overall risk incorporated these differences, even though they could not always be reflected adequately in the placement of an exposure into a particular category.

The chemicals, groups of chemicals, industrial processes, or occupational exposures were thus put into one of three groups:

Group 1

The chemical, group of chemicals, industrial process, or occupational exposure is carcinogenic to humans. This category was used only when there was sufficient evidence from epidemiological studies to support a causal association between the exposure and cancer.

Group 2

The chemical, group of chemicals, industrial process, or occupational exposure is probably carcinogenic to humans. This category includes exposures for which, at one extreme, the evidence of human carcinogenicity is almost "sufficient," as well as exposures for which, at the other extreme, it is inadequate. To reflect this range, the category was divided into higher (Group A) and lower (Group B) degrees of evidence. Usually, category 2A was reserved for exposures for which there was at least limited evidence of carcinogenicity to humans. The data from studies in experimental animals played an important role in assigning studies to category 2, and particularly those in Group B; thus, the combination of sufficient evidence in animals and inadequate data in humans usually resulted in a classification of 2B.

In some cases, the Working Group considered that the known chemical properties of a compound and the results from short-term tests allowed its transfer from Group 3 to 2B or from Group 2B to 2A.

Group 3

The chemical, group of chemicals, industrial process, or occupational exposure cannot be classified as to its carcinogenicity to humans.